PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12Q 1/68, C12N 15/85, 9/02, C07K 16/40, A61K 39/395, C12N 5/10, A61K 48/00, 31/70, G01N 33/573, A01K 67/027

A2

(11) International Publication Number: **WO 00/09754**

(43) International Publication Date: 24 February 2000 (24.02.00)

(21) International Application Number: PCT/US99/18387

(22) International Filing Date: 12 August 1999 (12.08.99)

(30) Priority Data:

60/096,520	14 August 1998 (14.08.98)	US
Not furnished	5 August 1999 (05.08.99)	US

(71) Applicant: JOHNSON & JOHNSON CONSUMER COMPANIES, INC. [US/US]; Grandview Road, Skillman, NJ 08558 (US).

(72) Inventors: STENN, Kurt; 46 Bayard Lane, Princeton, NJ 08540 (US). PROUTY, Stephen, M.; 236 Windsor Way, Doylestown, PA 18901 (US). PARIMOO, Satish; 6 Betts Trail, Bridgewater, NJ 08807 (US). ZHANG, Lin; Apartment 73, 43 Farm Road, Somerville, NJ 08875 (US).

(74) Agents: CIAMPORCERO, Audley, A. et al.; Johnson & Johnson, One Johnson & Johnson Plaza, New Brunswick, NJ 08933–7003 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: HUMAN STEAROYL–CoA DESATURASE–RELATED COMPOSITIONS AND METHODS FOR TREATING SKIN DISORDERS

(57) Abstract

This invention provides a nucleic acid molecule encoding human stearoyl–CoA desaturase, and isolated protein encoded thereby. This invention also provides a method of diagnosing a human subject for a skin disorder characterized by an abnormal level of stearoyl–CoA desaturase expression. This invention further provides a method for determining whether an agent increases the expression level of human stearoyl–CoA desaturase in skin cells already expressing same. This invention further provides methods for determining whether an agent increases or decreases the expression level or activity of human stearoyl–CoA desaturase in skin cells already expressing same. This invention still further provides pharmaceutical compositions for treating human skin disorders characterized by abnormal stearoyl–CoA desaturase expression and/or activity, and related methods of treatment. Finally, this invention provides related antibodies, gene therapy vectors, and transgenic mice.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

HUMAN STEAROYL-CoA DESATURASE-RELATED COMPOSITIONS AND METHODS FOR TREATING SKIN DISORDERS

Field of the Invention

This invention relates to the diagnosis and treatment of skin disorders characterized by abnormal stearoyl-CoA desaturase expression and activity. The invention also relates to various means of identifying agents useful for treating such disorders.

Background of the Invention

Mammalian Fatty Acid Desaturases Generally

Fatty acid desaturases ("FAD's") are enzymes that catalyze the insertion of a double bond in fatty acids. In mammals, there appear to be two distinct families of fatty acid desaturases. Stearoyl-CoA desaturase-1 ("SCD1") (Strittmatter, et al. 1974; Lippiello, et al. 1979), a key regulatory enzyme of unsaturated fatty acid biosynthesis, belongs to the first such family. This enzyme introduces a cis-double bond at the delta-9 position of fatty acyl-CoA's to produce palmitoleoyl and oleoyl-CoA.

Mammalian fatty acid desaturases are structurally similar to each other. They are integral membrane, iron-containing enzymes that catalyze the NADH- and O_2-dependent formation of double bonds into methylene-interrupted fatty acyl chains. Various forms of mammalian stearoyl-CoA desaturase ("SCD") have been isolated from rat, mouse, human, bovine (St. John, et al. 1991), ovine, porcine, and hamster. The coding

regions of mouse, human and rat SCD sequences show over 80% nucleotide sequence identity. They apparently share a similar exonic structure, but differ markedly in upstream regulatory regions (Mihara 1990).

Stearoyl-CoA Desaturase and its Mechanism of Action

Stearoyl-CoA desaturase is responsible for the production of unsaturated fatty acids, which are required for energy and lipid metabolism, membrane structure, and signal transduction. The expression of stearoyl-CoA desaturase in the skin suggests an important role for unsaturated fatty acids in skin homeostasis, and specifically in the function of the pilosebaceous unit and the eccrine sweat gland.

SCD is the enzyme responsible for the committed step in unsaturated fatty acid ("UFA") synthesis, and as such is the key point of metabolic control in this pathway. SCD catalyzes the insertion of a double bond between C9 and C10 of its preferred substrates, palmitic and stearic acid. UFA's are important for three major reasons. UFA's are key components of cellular membranes. Triglycerides contain UFA's and are a major component of energy metabolism. Finally, UFA's play an important role in stimulating lipid-activated kinases during signal transduction. In the mouse, the SCD gene family was previously thought to contain 2 members: SCD1 ("M-SCD1") in adipose tissue and liver, and SCD2 ("M-SCD2") in brain.

A critical step in the biosynthesis of unsaturated fatty acids is the introduction of the first cis-double bond in the Δ9 position (between carbons 9 and 10)

(Ntambi 1995). The iron-containing stearoyl-CoA desaturase enzyme catalyzes this oxidative step. In this reaction, electrons flow from NADPH through NADH-cytochrome b5 reductase to cytochrome b5. Cytochrome b5 is the direct electron donor to the desaturase. Stearoyl-CoA desaturase is believed to utilize iron in an oxidative-reduction reaction transferring electrons to molecular oxygen with the production of H_2O (Strittmatter, et al. 1974). The rate-limiting step in this reaction is at the desaturase. It is this factor which is cyanide-sensitive and limits the overall desaturation rate (Oshino 1972; Oshino, et al. 1972). Acyl CoA derivatives of fatty acids containing 12 to 19 carbon residues are required for desaturase activity. (Enoch, et al. 1976). Shorter chain acyl CoA derivatives, free CoA and free fatty acids do not appear to bind to the enzyme (Enoch, et al. 1976). Additionally, SCD activity is affected by metal ions (Wahle, 1975).

Although SCD catalyzes the cis-desaturation of a spectrum of methylene-interrupted fatty acyl CoA substrates, the preferred substrates are palmitate (C16) and stearate (C18). Palmitate and stearate are converted into palmitoleoyl-CoA and oleoyl-CoA, respectively, by the enzyme. Palmitoleic and oleic acids are the major unsaturated fatty acid constituents of phospholipids and triacylglycerols. The former is central to membrane structure and the latter to the lipid store found in adipocytes. The ratio of stearic to oleic acid is one of the factors influencing cell membrane fluidity.

Mouse Stearoyl-CoA Desaturases

Two mouse SCD genes have been identified, and designated M-SCD1 and M-SCD2. M-SCD1 cDNA was isolated from 3T3-L1 preadipocytes which had been shown to express M-SCD1 upon differentiation (preadipocytes into adipocytes) (Ntambi, et al. 1988). The M-SCD1 gene encodes a 4.9kb mRNA. The predicted amino acid sequence of the mouse 3T3 adipocyte SCD exhibits 92% similarity to rat liver SCD1. There is also a high degree of nucleotide sequence identity between mouse and rat mRNA's in their unusually long (3.5kb) 3' untranslated regions ("UTR's") (Ntambi, et al. 1988). The SCD1 gene spans 15 kb and contains 6 exons and 5 introns.

The 5' end of the SCD-1 gene shows a canonical TATA box (Ntambi, et al. 1988). A region similar to the binding site for the nuclear transcription factor, Sp1, is present. Upstream of the transcription initiation site are regions homologous to the fat-specific transcription element FSE2. Core consensus sequences for cAMP and glucocorticoid regulatory elements are present (Ntambi, et al. 1988). In the promoter region, the PPAR receptor localizes to an AGGTCA consensus sequence between base pairs -664 to -642 (Miller, et al. 1996). C/EBPα can bind to the M-SCD1 promoter and activate transcription during the late stage of adipocyte differentiation (Christy, et al. 1989).

The M-SCD2 gene spans approximately 15 kb and, like M-SCD1 and rat SCD, consists of 6 exons and 5 introns (Kaestner, et al. 1989; Mihara 1990). The

promoter regions for M-SCD2 have also been characterized (Kaestner, et al. 1989). The 5' end of M-SCD2 lacks a typical 5' TATA box, but has two CCAAT boxes. The M-SCD2 promoter contains a region (located between nucleotides -54 to -201) which shows a 77% sequence identity to a region in the M-SCD1 promoter. It contains a site similar to the nuclear transcription factor, Sp1, and an element with homology to the core consensus sequence of the glucocorticoid regulatory element (Kaestner, et al. 1989).

From studies of genomic blots, the prediction that other forms of the SCD family might exist in the rat and mouse have been suggested in the literature but, heretofore, not pursued (Mihara 1990; Ntambi 1995).

The SCD genes have tissue-specific expression patterns. Under normal dietary states, M-SCD1 mRNA's are expressed constitutively in adipose, but not hepatic, tissue. Their expression is induced in liver in response to a fat-free, high-carbohydrate diet. M-SCD2 mRNA's are constitutively expressed in brain and induced in kidney, lung, spleen and adipose tissue in response to a high carbohydrate diet, but not expressed in liver under either condition (Kaestner, et al. 1989). It is notable that in vivo, the desaturases are short-lived, having a half-life only a few hours (Oshino, et al. 1972; Ozols 1997).

The *Asebia* Mutation in Mice

The *asebia* mutation was first described in mice by Gates, et al. (1965). The mutation arose as an autosomal recessive trait in the BALB/c mouse strain.

Prominent elements of the phenotype include early loss of hair, scaly skin, and sebaceous glands that fail to fully develop. Since the meibomian glands are also hypoplastic, these mice also have eye problems. The ocular findings have been described as eye inflammation, photophobia and, finally, scarring of the eyelid with resultant blindness. Histologically (Josefowicz, et al. 1978a, 1978b, 1978c), besides showing hypoplastic sebaceous glands, their skin also shows epidermal hyperplasia and excessively long anagen hair follicles. The hair follicle anagen phase (i.e. growth phase) is prolonged compared to the wild-type mouse. Foreign body and chronic inflammatory reactions are present in the dermis. With age, the dermis becomes increasingly scarred with permanent loss of pilosebaceous structures. Laboratory studies of *asebia* mouse epidermis show that the water permeability barrier is reduced.

According to thin-layer chromatography tests, skin surface lipids are abnormal. Wilkinson, et al. (1966) found that there are alterations in the balance of waxy esters, fatty acids, and cholesterol esters. Transplantation studies suggest that the genetic abnormality is expressed in the epidermis and not the dermis (Pennycuik, et al. 1986).

Human Stearoyl-CoA Desaturase

Using primers based on the rat cDNA sequences, a human stearoyl-CoA desaturase cDNA of 712 bp (not encoding the full-length ORF) was identified by PCR from adipose tissue (Li, et al. 1994). This form is referred to herein as human adipose SCD ("HA-SCD").

From the determined sequence, it was seen that at the nucleotide level, the homology to the various mouse and rat SCD's is between 80-84%. The similarity in deduced peptide sequence between human and mouse SCD's is approximately 93%. RNAse protection demonstrates either no, little, or variable expression in normal esophagus, colon, and liver, respectively. Increased expression is seen in tumors derived from these three tissues.

A human cDNA from liver is present in the database (Wisconsin Package Version 9.1, Genetics Computer Group, GCG, Madison, Wisc.: accession number: Y13647) that contains part of the 5' and 3' UTR's and the complete ORF of SCD. This form is referred to herein as human liver SCD ("HL-SCD"). At the nucleotide level, the identity of HL-SCD to HA-SCD is 98.6% (over the known sequence of HA-SCD); to M-SCD1 is 76.2%; and to mouse SCD2 is 75.1%. At the amino acid level, identity of HL-SCD to HA-SCD is 98.7%, and is 83.9% to the M-SCD1.

Biological Effects of Fatty Acid Desaturation

Desaturases are enzymes that produce a variety of UFA's. UFA's are important for biological systems in the following ways: (1) as intermediates in lipid and energy metabolism (Neely, et al. 1974); (2) as components of triglycerides, which are a major form of cellular energy storage and the major component of circulating lipoprotein particles (Rosseneu, et al. 1995) (oleate is the major storage form of fatty acids in human adipose tissue (Berry 1997)); (3) as regulators of membrane fluidity; and (4) as components of signal transduction pathways.

In order to maintain the proper function of cellular membranes, there must be tight regulation of membrane fluidity (Kates, et al. 1984). This is achieved by the ratio of saturated fatty acid ("SFA") to UFA. This ratio is largely controlled by enzymes which produce these lipids, which are fatty acid synthase and fatty acid desaturase, respectively. Δ9 desaturase activity increases when organisms are exposed to low temperature, in order to increase the amount of desaturated fatty acids in the cellular membranes. These desaturated fatty acids increase fluidity and thereby prevent cold-induced rigidification (Kasai, et al. 1976; Tiku, et al. 1996).

Membrane fluidity has also been related to cell-cell signalling and cancer formation. The degree of membrane fluidity can affect the function of receptors (Clandinin, et al. 1991), and increased concentrations of membrane UFA's have been detected in tumor cells, suggesting a role in neoplasia (Li, et al. 1994). Many tumors show altered fatty acid profiles, especially an increase in oleate (Hrelia, et al. 1994). This change in membrane fluidity may confer changes in response to cell-cell and/or intra-cellular signalling. Indeed, the expression of high levels of yeast SCD in mammalian tumor cells increases membrane fluidity and greatly increases tumor necrosis factor signaling (Gyorfy, et al. 1997).

Through studies of signalling molecules that are regulated by lipids, UFA's are implicated in the regulation of cellular growth and differentiation. UFA's can co-activate various isoforms of protein

kinase C (PKC) (Shinomura, et al. 1991). They can also alter the subcellular localization of PKC (Diaz-Guerra, et al. 1991), a process known to activate this enzyme. PKC plays an important role in the normal growth and differentiation of epidermal cells (Dlugosz, et al. 1993) and hair follicles (Harmon, et al. 1995), and is implicated in the pathogenesis of psoriasis (Rassmussen, et al. 1993; Wevers, et al. 1992). Intracellular free UFA's can be generated by receptor-stimulated phospholipase A action on membrane phospholipids (Liscovitch, et al. 1994).

During adipocyte differentiation in vitro, the transcription of M-SCD1 is activated during the early phase, suggesting a role for UFA's in the regulation of adipocyte differentitation (Casimir, et al. 1996).

UFA's are now recognized as activators of gene expression via transcription factors that bind to UFA's, such as peroxisome proliferator-activated receptor (Bocos, et al. 1995) and fatty acid-activated receptor (Ailhaud, et al. 1995). Phosphatidylinositol (PtdIns)-3,4,5-triphosphate (P3) is a lipid second messenger that is formed by the phosphorylation of the 3 position of the inositol ring of PtdIns-4,5-bisphosphate (located in plasma membranes) by the receptor-activated phosphatidylinositol-3-OH kinase [PI(3)K]. PtdIns-3,4,5-P3 activates downstream kinases PDK1, PDK2, and PKB by recruiting these enzymes to the plasma membrane (Alessi, et al. 1998). It has been shown that the most effective form of PtdIns-3,4,5-P3 for activating PKB is that with oleate at the 2 position of the phospholipid.

The biological processes regulated by PI3 kinase and PKB pathways are pleiotropic, and include membrane trafficking, adhesion, cell growth, and survival (Toker, et al. 1997). Recently, Cadena, et al. (1997) isolated a divergent member of the fatty acid desaturase gene family, termed "MLD" for membrane fatty acid lipid desaturase, from the human HeLa cell line. It was shown that it specifically interacts with the cytoplasmic tail of the epidermal growth factor receptor, and is thought to play a role in the biosynthesis of the receptor, thus indirectly controlling its function (Cadena, et al. 1997).

Skin Pathology Related to Fatty Acid Desaturation

The skin is recognized as a lipid-rich organ, the proper function of which depends on the integrity of lipid metabolism. It has long been known that essential fatty acid deficiency has profound effects on the skin. Prominent effects include scaling of skin and increased trans-epidermal water loss (Holman 1993). It is notable that the *asebia* mouse also manifests these changes.

Atopic dermatitis, a chronic inflammatory skin disease, is ameliorated by the administration of γ-linolenic acid, indicating involvement of fatty acid metabolism in its pathogenesis (Youn, et al. 1998). The integrity of the stratum corneum is heavily dependent on the lipid composition of the corneocytes, which act to promote hydrophobicity as well as maintain adhesion (Chen, et al. 1996). Similarly, the maintenance of adhesion between the cuticle and the cortex of the hair shaft is dependent on specialized

fatty acids, whose defects are manifest in hair from patients with Maple Syrup Urine Disease (Jones, et al. 1996).

As stated above, the pilosebaceous unit is sensitive to alterations in fatty acid composition. This is futher demonstrated by data indicating that local deficiency of linolenic acid in the sebaceous gland leads to proliferation of the keratinocytes lining its duct. This in turn leads to the formation of comedones, the precursor to acne vulgaris (Downing, et al. 1986).

Alterations of lipid transport have dramatic effects on skin, as evidenced by the phenotype of the ApoC1 over-expressing transgenic mouse (Jong, et al. 1998). This animal has scaly skin, loss of hair, and hypoplastic sebaceous glands, likely due to decreased delivery of free fatty acids to the skin. The striking similarity of this phenotype to that of *asebia* suggests that the two mutations may invole genes on the same metabolic and/or signalling pathway. Furthermore, the human disease, ichthyosis follicularis, manifests strikingly similar features to the *asebia* mouse, these being loss of hair, hypoplastic sebaceous glands, scaly skin, and photophobia (Eramo, et al. 1985).

Pathology of Hair Growth

Hair matrix keratinocytes are the highly proliferative cells that give rise to the shaft and sheath of the hair. They are termed transient amplifying stem cells to indicate that their proliferation correlates with the growth phase of the

hair, and that their quiescence correlates with the resting phase (i.e., no growth) of the hair follicle (Cotsarelis, et al. 1990). Hypertrichosis (Olsen, 1994) and hirsutism (Hughes, Jr. 1994) are diseases of the hair follicle that result from excessive growth. Alopecia encompasses several distinct diseases that result in a lack of hair growth (Rietscel 1996).

Summary of the Invention

This invention provides a nucleic acid molecule encoding the human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof.

This invention also provides a nucleic acid molecule which, under suitable conditions, specifically hybridizes to a nucleic acid molecule encoding the human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof.

This invention further provides a method of diagnosing a human subject for a skin disorder characterized by an abnormal level of stearoyl-CoA desaturase expression, which comprises (i) obtaining a sample of skin mRNA from the subject; (ii) contacting the sample so obtained with an excess of the instant labeled nucleic acid molecule under conditions permitting hybridization of the labeled nucleic acid molecule with stearoyl-CoA desaturase mRNA present in the sample; (iii) removing un-hybridized labeled nucleic acid molecule from the sample; (iv) quantitatively determining the amount of hybridized labeled nucleic acid molecule present in the sample; and (v) comparing the amount determined in step (iv) with the amount determined using a skin mRNA sample from a normal human subject, a difference in these amounts being correlative of an abnormal level of stearoyl-CoA desaturase expression in the skin of the subject being diagnosed.

This invention further provides an isolated human stearoyl-CoA desaturase encoded by the instant nucleic acid molecule.

This invention still further provides a eukaryotic cell line which expresses human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof, wherein the cell is transfected with an expression vector encoding the desaturase.

This invention provides a method for determining whether an agent increases the expression level of human stearoyl-CoA desaturase in skin cells already expressing same, which comprises the steps of (i) contacting the agent under suitable conditions with a eukaryotic cell line expressing human stearoyl-CoA desaturase at a known level; and (ii) determining whether the stearoyl-CoA desaturase expression level increases after cellular contact with the agent, thereby determining whether the agent increases the expression level of human stearoyl-CoA desaturase in skin cells already expressing same.

This invention also provides a method for determining whether an agent decreases the expression level of human stearoyl-CoA desaturase in skin cells already expressing same, which comprises the steps of (i) contacting the agent under suitable conditions with a eukaryotic cell line expressing human stearoyl-CoA desaturase at a known level; and (ii) determining whether the stearoyl-CoA desaturase expression level decreases after cellular contact with the agent, thereby determining whether the agent decreases the

expression level of human stearoyl-CoA desaturase in skin cells already expressing same.

This invention further provides a method for determining whether an agent decreases the activity of human stearoyl-CoA desaturase in skin cells, which comprises the steps of (i) contacting the agent under suitable conditions with human stearoyl-CoA desaturase having a known level of activity; and (ii) determining whether the desaturase activity decreases after contact with the agent, thereby determining whether the agent decreases human stearoyl-CoA desaturase activity in skin cells.

This invention further provides a method for determining whether an agent increases the activity of human stearoyl-CoA desaturase in skin cells, which comprises the steps of (i) contacting the agent under suitable conditions with human stearoyl-CoA desaturase having a known level of activity; and (ii) determining whether the desaturase activity increases after contact with the agent, thereby determining whether the agent increases human stearoyl-CoA desaturase activity in skin cells.

This invention provides an antibody which specifically binds to human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof, and thereby inhibits the activity thereof.

This invention also provides a pharmaceutical composition for treating a human skin disorder characterized by an excess of stearoyl-CoA desaturase

activity, which comprises a therapeutically effective amount of the instant antibody and a pharmaceutically acceptable carrier for use in topical administration.

This invention further provides an expression vector suitable for use in gene therapy, which vector encodes a nucleic acid molecule capable of specifically inhibiting the expression of human skin stearoyl-CoA desaturase.

This invention further provides a pharmaceutical composition for treating a human skin disorder characterized by an excess of skin stearoyl-CoA desaturase activity, which comprises the instant expression vector, and a pharmaceutically acceptable carrier for use in topical administration.

This invention still further provides a method for treating a human subject afflicted with a skin disorder characterized by an excess of stearoyl-CoA desaturase activity, which comprises topically administering to the subject a therapeutically effective dose of the instant SCD activity-reducing pharmaceutical composition.

This invention provides an SCD-encoding DNA expression vector suitable for use in gene therapy.

This invention also provides a pharmaceutical composition for treating a human skin disorder characterized by insufficient skin stearoyl-CoA desaturase activity, which comprises the instant SCD-encoding expression vector, and a pharmaceutically acceptable carrier for use in topical administration.

This invention further provides a method for treating a human subject afflicted with a skin disorder characterized by insufficient stearoyl-CoA desaturase activity, which comprises topically administering to the subject a therapeutically effective dose of the instant SCD activity-increasing pharmaceutical composition.

This invention provides the instant antibody labeled with a detectable marker. This invention also provides an antigen suitable for use in generating the instant antibody, which comprises at least a portion of human stearoyl-CoA desaturase.

This invention also provides a method of producing the instant antibody, which comprises the steps of administering to a suitable animal an antigenic portion of human stearoyl-CoA desaturase, and after a suitable length of time, isolating the antibody generated by the animal against the antigenic portion so administered.

This invention further provides a method of diagnosing a human subject for a skin disorder characterized by an abnormal level of stearoyl-CoA desaturase expression, which comprises (i) obtaining a stearoyl-CoA desaturase-containing sample from the subject's skin; (ii) contacting the sample so obtained with an excess of the instant antibody under conditions permitting binding of the antibody with stearoyl-CoA desaturase present in the sample; (iii) removing un-bound antibody from the sample; (iv) quantitatively determining the amount of bound antibody present in the sample; and (v) comparing the amount determined in step

(iv) with the amount determined using a skin stearoyl-CoA desaturase sample from a normal human subject, a difference in these amounts being correlative of an abnormal level of stearoyl-CoA desaturase expression in the skin of the subject being diagnosed.

Finally, this invention provides a transgenic mouse whose skin cells do not express any gene encoding mouse skin stearoyl-CoA desaturase having the amino acid sequence shown in Figure 1 or 2, or any polymorphism thereof.

Brief Description of the Figures

Figure 1 shows the sense strand sequence (mRNA sequence) of M-SCD3 cDNA obtained after sequencing 5' RACE cDNA clone from mouse skin mRNA. The sequence corresponding to the coding sequence (i.e., the protein sequence) is underlined. The M-SCD3-specific in situ hybridization (ISH) probe is boxed.

Figure 2 shows the sense strand sequence (mRNA sequence) of M-SCD4v1 cDNA from two overlapping novel cDNA clones obtained by screening a mouse skin cDNA library with the M-SCD3 probe. The sequence corresponding to the coding sequence (protein sequence) is underlined. The boxed sequence corresponds to the 3' half of the ISH probe for M-SCD4v2 that has 100% identity with M-SCDv1.

Figure 3 shows the homology between M-SCD3 cDNA sequence and the M-SCD4v1 cDNA sequence. The regions of homology are shown with vertical lines. The protein-coding sequence is underlined. The boxed sequence on the M-SCD4v1 sequence corresponds to the 3' half of the ISH probe for M-SCD4v2 that has 100% identity with M-SCDv1. The bases that are boxed on the M-SCD3 sequence correspond to the M-SCD3-specific ISH probe.

Figure 4 shows a comparison of the four mouse SCD cDNA sequences, i.e., M-SCD's 1, 2, 3 and 4. The M-SCD4 cDNA sequence is that of M-SCD4v1. The protein-coding region is underlined. The first nucleotide beginning significant homology between a sequence and one or more of the other sequences is boxed and ends with the coding region.

Figure 5 shows the deduced protein sequence from the M-SCD3 sense strand cDNA. The sequence is from amino acids 1-289. The single code designation of amino acids is the standard biochemical single code designation for amino acids from the GCG computer program of Wisconsin Package (Genetics Computer Group, Madison, Wisconsin).

Figure 6 shows the complete protein-coding sequence of mouse skin SCD4v1 (359 amino acids) deduced from its cDNA sequence.

Figure 7 shows a comparison of mouse protein sequences derived from four SCD genes. The amino acid residues which are not common in all the four protein sequences are underlined. The boxed histidine residues are conserved in evolution from yeast to mammals.

Figure 8 shows the cDNA sequence (sense strand) and protein sequence of human SCD obtained from skin. The ORF extends from bp 229 to bp 1308 and encodes a predicted protein sequence of 359 amino acids. The boxed sequence corresponds to the human ISH probe.

Figure 9 shows a comparison between human skin cDNA and database-deposited human liver cDNA encoding SCD. Identical bases are indicated with vertical lines. All bases differing between skin and liver are indicated with boxes. The protein-coding sequence is underlined.

Figure 10 shows a comparison between human skin cDNA and database-deposited human adipose cDNA. Identical bases are indicated with vertical lines. All bases

differing between skin and adipose are indicated with boxes. The protein-coding sequence is underlined.

Figure 11 shows a comparison of predicted amino acid sequences derived from human skin SCD, human liver SCD, and human adipose SCD. Amino acid differences are boxed. The conserved histidine residues are underlined. The adipose sequence does not contain the complete ORF.

Figure 12 shows the cDNA sequence homology (5' end) of sense strands of the two mouse skin SCD4 variant species. The regions of homology are connected by vertical bold lines. The protein-coding region is underlined. The boxed sequence corresponds to an ISH probe that recognizes both variant forms (v1 and v2) of M-SCD4.

Figure 13 shows the cDNA sequence homology (3' end) of sense strands of the two mouse skin SCD4 variant species. The regions of homology are connected by vertical bold lines. The region of a 6-nucleotide difference at the 3' end is boxed. The protein-coding region is underlined.

Detailed Description of the Invention

This invention relates to the diagnosis and treatment of skin disorders characterized by abnormal stearoyl-CoA desaturase expression and activity. The invention also relates to various means of identifying agents useful for treating such disorders. Underlying this invention is the surprising discovery that stearoyl-CoA desaturase in mice and, more importantly, in humans, is expressed in skin. Skin is a lipid-rich organ, and many skin disorders such as atopic dermatitis and acne involve lipid imbalances. Stearoyl-CoA desaturase - discovered here to be expressed in skin - now serves as an important new therapeutic target and diagnostic indicator for certain lipid-related skin disorders.

More specifically, this invention provides a nucleic acid molecule encoding the human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof. A polymorphism of the SCD whose sequence is shown in Figure 8 means any naturally occurring human SCD whose amino acid sequence varies therefrom due to one or more intra-species mutations.

The instant SCD-encoding nucleic acid molecule can be any type of nucleic acid molecule, such as mRNA and DNA. In the preferred embodiment, the instant nucleic acid molecule is a DNA molecule. DNA molecules envisioned in this invention include, by way of example, cDNA molecules, which can optionally be isolated molecules. In the preferred embodiment, the nucleic acid molecule is a cDNA molecule comprising the

sequence shown in Figure 8. Moreover, the instant SCD-encoding DNA molecule can be any form of DNA permitting the expression thereof, or any form of DNA, such as an insert, which serves as a precursor to a form permitting expression. In the preferred embodiment, the DNA molecule is in an expression vector.

Stearoyl-CoA desaturase is alternatively referred to herein as "SCD". In addition, human SCD is alternatively referred to as "H-SCD" or "HS-SCD", and mouse SCD1, SCD2, SCD3 and SCD4 are alternatively referred to as "M-SCD1", "M-SCD2", "M-SCD3" and "M-SCD4", respectively.

It is also important to note the following points. First, M-SCD3 and M-SCD4 are novel genes discovered as disclosed hereinbelow. Second, the H-SCD sequence alternatively identified herein either as "skin H-SCD" or "HS-SCD", is novel and is expressed in skin, as well as other tissues. Third, the terms "skin SCD", "HS-SCD", "liver SCD", "HL-SCD", "adipose SCD" and "HA-SCD" are intended solely to indicate the organ from which their respective SCD-encoding mRNA's were obtained, and not to indicate that these organs are the only ones in which those SCD's are respectively expressed. Finally, the known H-SCD's identified herein as HA-SCD and HL-SCD not only differ in sequence from the instant HS-SCD, but, based on experimental discrepancies, may be polymorphisms of HS-SCD, erroneously sequenced non-HS-SCD's, or non-human SCD's entirely. Thus, it is possible that the instant HS-SCD is the first human SCD gene ever identified. In any event, the terms "human stearoyl-CoA desaturase", "H-SCD" and "HS-SCD", as they relate to the instant invention, shall mean only the

protein whose sequence is provided in Figure 8, and polymorphisms thereof. HL-SCD and HA-SCD, on the other hand, are included herein solely for the sake of comparison to the instant HS-SCD.

This invention also provides a nucleic acid molecule which, under suitable conditions, specifically hybridizes to a nucleic acid molecule encoding the human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof. Ideally, this nucleic acid molecule, which is preferably a DNA molecule, functions as a probe to detect and/or quantitate human stearoyl-CoA desaturase-encoding nucleic acid molecules in a sample. Accordingly, in the preferred embodiment, the molecule is labeled with a detectable marker.

Methods and suitable conditions for hybridizing detectable nucleic acid probes to the nucleic acid molecules being detected are well known in the art (Farrell Jr., 1993). As used herein, the instant nucleic acid molecule "specifically hybridizes" with the H-SCD sequence if it hybridizes to that sequence, but not to any other SCD sequence to a significant degree. Ideally, the instant nucleic acid molecule hybridizes to the H-SCD-encoding molecule at least 10-fold more strongly than to any other human mRNA or cDNA. Detectable markers such as radiolabels and fluorescent labels, and methods of using same to label nucleic acid molecules, are well known in the art (Farrell Jr., 1993). In one embodiment, the condition suitable for hybridizing is a stringent hybridizing condition described in Sambrook, J., et al. (1989).

This invention further provides a method of diagnosing a human subject for a skin disorder characterized by an abnormal level of stearoyl-CoA desaturase expression, which comprises (i) obtaining a sample of skin mRNA from the subject; (ii) contacting the sample so obtained with an excess of the instant labeled nucleic acid molecule under conditions permitting hybridization of the labeled nucleic acid molecule with stearoyl-CoA desaturase mRNA present in the sample; (iii) removing un-hybridized labeled nucleic acid molecule from the sample; (iv) quantitatively determining the amount of hybridized labeled nucleic acid molecule present in the sample; and (v) comparing the amount determined in step (iv) with the amount determined using a skin mRNA sample from a normal human subject, a difference in these amounts being correlative of an abnormal level of stearoyl-CoA desaturase expression in the skin of the subject being diagnosed.

Skin disorders that can be diagnosed by the instant method include, for example, skin cancer, acne, atopic dermatitis, alopecia, hirsutism, and hypertrichosis. Methods for obtaining tissue-specific mRNA samples (e.g. skin mRNA), conditions permitting hybridization therewith by the instant labeled nucleic acid molecule, and methods of quantitatively determining the amount of hybridization by the labeled molecule, are all well known in the art (Farrell Jr., 1993).

This invention further provides an isolated human stearoyl-CoA desaturase encoded by the instant nucleic acid molecule. In the preferred embodiment, the

isolated desaturase has the amino acid sequence shown in Figure 8.

In one embodiment, the "isolated" H-SCD protein is free of any other SCD protein. In the preferred embodiment, the isolated H-SCD protein is free of any other protein. Methods that can be used for making the H-SCD protein, such as recombinant protein production and transfected cell culturing, are well known (Sambrook, et al. 1989). Methods that can be used for isolating H-SCD protein, such as column chromatography and gel electrophoresis, are also well known (Sambrook, et al. 1989).

This invention further provides a eukaryotic cell line which expresses human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof, wherein the cell is transfected with an expression vector encoding the desaturase.

Suitable eukaryotic cell lines include, but are not limited to, yeast cells, insect cells and animal cells. Suitable animal cells include, but are not limited to HeLa cells, Cos cells, CV1 cells and various primary mammalian cells. Numerous mammalian cells may be used as hosts, including, but not limited to, the mouse fibroblast cell NIH-3T3 cells, CHO cells, HeLa cells, Ltk$^-$ cells and COS cells. In the preferred embodiment, the eukaryotic cell line is a mammalian cell line, ideally one comprising, or derived from, skin tissue cells. The eukaryotic cell lines may be transfected by methods well known in the art such as calcium phosphate precipitation, electroporation, lipofection, and microinjection.

This invention provides several methods of screening agents for therapeutic and prophylactic use in connection with SCD-related disorders. First, this invention provides a method for determining whether an agent increases the expression level of human stearoyl-CoA desaturase in skin cells already expressing same, which comprises the steps of (i) contacting the agent under suitable conditions with a eukaryotic cell line expressing human stearoyl-CoA desaturase at a known level; and (ii) determining whether the stearoyl-CoA desaturase expression level increases after cellular contact with the agent, thereby determining whether the agent increases the expression level of human stearoyl-CoA desaturase in skin cells already expressing same.

Second, this invention provides a method for determining whether an agent decreases the expression level of human stearoyl-CoA desaturase in skin cells already expressing same, which comprises the steps of (i) contacting the agent under suitable conditions with a eukaryotic cell line expressing human stearoyl-CoA desaturase at a known level; and (ii) determining whether the stearoyl-CoA desaturase expression level decreases after cellular contact with the agent, thereby determining whether the agent decreases the expression level of human stearoyl-CoA desaturase in skin cells already expressing same.

In the instant cell-based methods, the amount by which the H-SCD expression level is increased or decreased can be any quantifiable amount. In the preferred embodiment, this amount is at least a 50% increase or decrease in expression level. Methods

which can be used for quantitatively determining such increase or decrease include, for example, labeled probe hybridization with skin mRNA Northern blots, and are well known in the art (Farrell, Jr. 1993).

Also, in the instant cell-based methods, the eukaryotic cell line can be either (a) a cell line transfected with an expression vector encoding a human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof, or (b) a non-transfected cell line.

Third, this invention provides a method for determining whether an agent decreases the activity of human stearoyl-CoA desaturase in skin cells, which comprises the steps of (i) contacting the agent under suitable conditions with human stearoyl-CoA desaturase having a known level of activity; and (ii) determining whether the desaturase activity decreases after contact with the agent, thereby determining whether the agent decreases human stearoyl-CoA desaturase activity in skin cells.

Lastly, this invention provides a method for determining whether an agent increases the activity of human stearoyl-CoA desaturase in skin cells, which comprises the steps of (i) contacting the agent under suitable conditions with human stearoyl-CoA desaturase having a known level of activity; and (ii) determining whether the desaturase activity increases after contact with the agent, thereby determining whether the agent increases human stearoyl-CoA desaturase activity in skin cells.

In one embodiment of these methods, "activity of H-SCD" means the rate at which the SCD introduces a cis-double bond in its substrate palmitate to produce palmitoleoyl-CoA. Methods that can be used to quantitatively measure SCD activity include, for example, measuring thin layer chromatographs of SCD reaction products over time. This method and others methods suitable for measuring SCD activity are well known (Henderson, et al. 1992).

This invention also provides an antibody which specifically binds to human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof, and thereby inhibits the activity thereof.

The instant antibody can be a polyclonal antibody, a monoclonal antibody, or an SCD-binding fragment thereof. In one embodiment, the antibody is an isolated antibody, i.e., an antibody free of any other antibodies. The term "antibody" includes, by way of example, both naturally occurring and non-naturally occurring antibodies. Furthermore, the term "antibody" includes chimeric antibodies and wholly synthetic antibodies, and fragments thereof. Methods of making and isolating antibodies are well known in the art (Harlow, et al. 1988).

This invention further provides a pharmaceutical composition for treating a human skin disorder characterized by an excess of stearoyl-CoA desaturase activity, which comprises a therapeutically effective amount of the instant antibody and a pharmaceutically acceptable carrier for use in topical administration.

In this invention, topically administering the instant pharmaceutical compositions can be effected or performed using any of the various methods and delivery systems known to those skilled in the art. The topical administration can be performed, for example, transdermally and via topical injection.

Pharmaceutical carriers for topical administration are well known in the art, as are methods for combining same with active agents to be delivered. Examples of topical carriers and their uses are well known in the art (Ramchandani; Barry; Wenniger; Martindale's Parmacopoeia; U.S. Pharmacopeoia). The following dermal delivery systems, which employ a number of routinely used carriers, are only representative of the many embodiments envisioned for administering the instant composition.

Transdermal delivery systems include, for example, aqueous and nonaqueous gels, creams, multiple emulsions, microemulsions, liposomes, ointments, aqueous and nonaqueous solutions, lotions, aerosols, hydrocarbon bases and powders, and can contain excipients such as solubilizers, permeation enhancers (e.g., fatty acids, fatty acid esters, fatty alcohols and amino acids), and hydrophilic polymers (e.g., polycarbophil and polyvinylpyrolidone). In the preferred embodiment, the pharmaceutically acceptable carrier is a liposome or a transdermal enhancer. Examples of liposomes which can be used in this invention include the following: (1) CellFectin, 1:1.5 (M/M) liposome formulation of the cationic lipid N,N^{I},N^{II},N^{III}-tetramethyl-N,N^{I},N^{II},N^{III}-tetrapalmityl-

spermine and dioleoyl phosphatidylethanolamine (DOPE) (GIBCO BRL); (2) Cytofectin GSV, 2:1 (M/M) liposome formulation of a cationic lipid and DOPE (Glen Research); (3) DOTAP (N-[1-(2,3-dioleoyloxy)-N,N,N-trimethyl-ammoniummethylsulfate) (Boehringer Manheim); and (4) Lipofectamine, 3:1 (M/M) liposome formulation of the polycationic lipid DOSPA and the neutral lipid DOPE (GIBCO BRL).

This invention still further provides an expression vector suitable for use in gene therapy, which vector encodes a nucleic acid molecule capable of specifically inhibiting the expression of human skin stearoyl-CoA desaturase. In one embodiment, the nucleic acid molecule is an anti-sense molecule which is complementary to, and specifically hybridizes with, at least a portion of human stearoyl-CoA desaturase mRNA.

In human gene therapy, anti-sense nucleic acid technology has been one of the major tools of choice to inactivate genes whose expression causes disease and is thus undesirable. The anti-sense approach, employing a nucleic acid molecule that hybridizes with an mRNA molecule encoding an undesirable gene, leads to the inhibition of gene expression. Methods of making and using anti-sense molecules against known target genes are known in the art (Agrawal, 1996).

This invention still further provides a pharmaceutical composition for treating a human skin disorder characterized by an excess of skin stearoyl-CoA desaturase activity, which comprises the instant

expression vector, and a pharmaceutically acceptable carrier for use in topical administration.

This invention also provides a method for treating a human subject afflicted with a skin disorder characterized by an excess of stearoyl-CoA desaturase activity, which comprises topically administering to the subject a therapeutically effective dose of the instant SCD activity-reducing pharmaceutical composition. In the preferred embodiment, the disorder is selected from skin cancer, hypertrichosis, and hirsutism.

Determining a therapeutically effective dose of the instant pharmaceutical composition can be done based on animal data using routine computational methods. In one embodiment, the therapeutically effective dose contains between about 1 µg and about 1 g of the instant activity-reducing vector. In another embodiment, the therapeutically effective dose contains between about 10 µg and about 100 mg of the vector. In a further embodiment, the therapeutically effective dose contains between about 100 µg and about 10 mg of the vector.

This invention provides an SCD-encoding DNA expression vector suitable for use in gene therapy. This invention also provides a pharmaceutical composition for treating a human skin disorder characterized by insufficient skin stearoyl-CoA desaturase activity, which comprises the instant SCD-encoding expression vector, and a pharmaceutically acceptable carrier for use in topical administration.

This invention further provides a method for treating a human subject afflicted with a skin disorder characterized by insufficient stearoyl-CoA desaturase activity, which comprises topically administering to the subject a therapeutically effective dose of the instant SCD activity-increasing pharmaceutical composition. In the preferred embodiment, the disorder is selected from acne, atopic dermatitis and alopecia.

In one embodiment, the therapeutically effective dose contains between about 1 µg and about 1 g of the instant SCD-encoding vector. In another embodiment, the therapeutically effective dose contains between about 10 µg and about 100 mg of the vector. In a further embodiment, the therapeutically effective dose contains between about 100 µg and about 10 mg of the vector.

This invention provides the instant antibody labeled with a detectable marker. This invention also provides an antigen suitable for use in generating the instant antibody, which comprises at least a portion of human stearoyl-CoA desaturase.

This invention also provides a method of producing the instant antibody, which comprises the steps of administering to a suitable animal an antigenic portion of human stearoyl-CoA desaturase, and after a suitable length of time, isolating the antibody generated by the animal against the antigenic portion so administered. Suitable animals include, by way of example, mammals such as mice, rabbits, goats, and monkeys. Suitable lengths of time for generating antibodies are well known in the art, and often include one or more

"booster" administrations subsequent to the initial antigen administration. Ideally, the antigen is administered along with an adjuvant according to well known methods.

This invention further provides a method of diagnosing a human subject for a skin disorder characterized by an abnormal level of stearoyl-CoA desaturase expression, which comprises (i) obtaining a stearoyl-CoA desaturase-containing sample from the subject's skin; (ii) contacting the sample so obtained with an excess of the instant antibody under conditions permitting binding of the antibody with stearoyl-CoA desaturase present in the sample; (iii) removing un-bound antibody from the sample; (iv) quantitatively determining the amount of bound antibody present in the sample; and (v) comparing the amount determined in step (iv) with the amount determined using a skin stearoyl-CoA desaturase sample from a normal human subject, a difference in these amounts being correlative of an abnormal level of stearoyl-CoA desaturase expression in the skin of the subject being diagnosed. Conditions required for antibody binding are well known. The antibody can be labeled or unlabeled. In one embodiment, the unlabeled antibody is quantitatively measured by means of a second, detectable antibody directed to the instant antibody.

This invention also provides a transgenic mouse whose skin cells do not express any gene encoding mouse skin stearoyl-CoA desaturase having the amino acid sequence shown in Figure 1 or 2, or any polymorphism thereof. This type of transgenic mouse is also known in the art as a "knock-out mouse", in that its

transgenic status inhibits the expression of an undesired gene. In the preferred embodiment, the transgenic mouse has operably integrated into its chromosomes a DNA sequence encoding human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof, which desaturase is expressed in the mouse's skin cells. Methods of making transgenic mice, including "knock-out" mice, are well known in the art (Hogan, et al. 1986).

Finally, for each embodiment of the human stearoyl-CoA desaturase-related nucleic acid molecules, compositions and methods provided herein, this invention also provides, *mutatis mutandis*, the corresponding embodiment of each of mouse SCD genes 3 and 4.

This invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that they are only illustrative of the invention as described more fully in the claims which follow thereafter. In addition, various publications are cited throughout this application. The disclosures of these publications are hereby incorporated by reference into this application to describe more fully the state of the art to which this invention pertains.

Experimental Details

Introduction: Discovery of Novel Mouse and Human SCD's

In an effort to identify and characterize novel genes involved in growth regulation of the pilosebacious unit ("PSU"), the molecules responsible for selected mouse PSU mutations were identified. There are approximately 48 rodent mutations that involve the skin and PSU (Sunberg 1994). Several of these mutations have been identified, such as *angora* (FGF5; Hebert, et al 1994), *waved-1* (TGFα: Mann, et al. 1993), *waved-2* (EGFR; Fowler, et al. 1995; Miettinen, et al. 1995), *nude* (whn; Nehls, et al. 1994), *balding* (DSG3; Koch, et al. 1997), and *hairless* (hairless; Ahmad, et al. 1998), and have provided new avenues of investigation for skin and PSU research.

The mutant mouse known as *asebia*, discussed in more detail hereinabove, was chosen as an experimental focal point. The most obvious phenotype is early loss of hair and hypoplasia of the sebaceous gland.

Previous work has demonstrated the importance of the sebaceous gland for proper development of the hair shaft (Williams, et al. 1997). This work has provided a possible explanation for the human hair disorder known as hair casts, in which the inner root sheath grows out with the hair shaft causing rough hair. Moreover, human acne is thought to result from aberrant changes in the sebaceous gland and the infundibulum of the hair follicle. Finally, several skin diseases including alopecia and hirsutism result from aberrant regulation of the hair growth cycle. Despite this information, an

understanding of the molecular events surrounding these conditions is unknown. It is in this light that a new molecular approach to these issues was sought by investigating the *asebia* mouse.

By using genomic mapping techniques, two genes in the PSU of *asebia* encoding SCD were identified. These genes, identified as M-SCD3 and M-SCD4, are located in the keratinocyte matrix cells of the hair follicle and in the sebaceous gland.

Following this discovery in mouse, a novel human SCD ("HS-SCD") was identified and characterized from skin tissue. Both the mouse and human SCD's of this invention are striking in that they are the first SCD's to be found expressed in skin.

Finding Candidates of the *asebia* Gene

Data from a backcross panel of about 600 mice led to the genomic localization of *asebia* trait as linked to a polymorphic genomic marker D19mit167. Further analysis of this genomic region led to identification of SCD as one of the possible candidate genes which may be altered in the *asebia (ab)* mice, leading to the observed pathological trait. Experimentation involving genomic DNA from mutant *ab* and normal mice suggested that SCD gene may be mutated in *ab* mice.

Using exon 5 reverse primer (from published data of SCD1, Ntambi et al., 1988), and 5' rapid amplification of cDNA ends ("RACE") cloning using a commercially available kit (Clontech, CA), a new cDNA sequence was identified after cloning of the 5' RACE

cDNA. Several race cDNA clones were sequenced, and one of the clones, 5α5, appeared novel since the sequence was highly homologous to SCD1, yet different in both coding and 5'non-coding regions. This clone was hence designated as M-SCD3. Using this clone as a probe, a commercially available mouse skin cDNA library was screened (Stratagene, CA) to identify additional novel SCD genes which might be expressed in skin.

Several cDNA clones were isolated, and from those clones, yet another gene was identified which is expressed in skin. Since this sequence was also unique in itself, this gene was designated SCD4. The SCD4 sequence was derived from overlapping sequences of two clones - 15g and 7d. A variant of the SCD4 sequence was identified after sequencing another clone - clone 5a. The former is identified herein as "SCD4v1" (variant 1) and the latter (derived from 5a clone) as "SCD4v2" (variant 2).

Nucleotide sequence analysis of M-SCD3 cDNA

The entire sequence of M-SCD3 clone (up to exon 5) is shown in Figure 1. An open reading frame for protein starts from nucleotide 285, and that part of the sequence is underlined. In addition to the coding sequence, it has 284 nucleotides of 5' non-coding sequence. Sequence comparison with the known M-SCD1 indicates that ~145 nucleotides at the 5' end of SCD3 are unique, whereas the rest of the sequence is highly homologous to the mouse SCD1 sequence (~99% identity). However, sequence comparisons with the known mouse SCD2 showed that the region of dissimilarity (unique 5' end of SCD3) stretched down to about 280 nucleotides. The

region downstream of 280 nucleotides of SCD3, which encompasses the protein-coding region, shows only ~90% identity with the SCD2 sequence. Hence, while the region of homology between SCD3 and SCD1 includes both the protein-coding region and a part of 5' non-coding region, the identity between SCD3 and SCD2 is limited to the protein-coding region of the genes.

Nucleotide sequence analysis of M-SCD4 cDNA

The nucleotide sequence of one of the isoforms of SCD4, SCD4v1, is shown in Figure 2. The sequence encompasses the entire protein-coding sequence (underlined) with 317 and 179 nucleotides of 5' and 3' UTR, respectively. Sequence comparison with the M-SCD1 sequence reveals that that the region of homology is limited to only the protein-coding sequence (underlined) (~91%identity), with no significant homology in either 5' or 3' non-coding regions. The sequence comparison with the M-SCD2 cDNA sequence again indicated a homologous region limited to the protein-coding segment (underlined), with sequence identity of about 88%. After the two novel sequences, SCD3 and SCD4, were compared to each other (Figure 3), they were shown to share an overall identity of about 77%, with maximum homology in their coding sequence (~90%). A search of the GenEMBL database also revealed homology between the M-SCD4 cDNA sequence and the FAR17-C cDNA sequence isolated from hamster flank organ. These two sequences share an overall identity of ~85%. The two sequences also showed regions of significant homology within 5' non-coding and 3' non-coding regions.

Variant Forms of M-SCD4 gene

Two distinct clones of SCD4 cDNA were identified, as indicated by unique sequences in both the 5' untranslated region (Figure 12) and 3' untranslated region (Figure 4). These two species may be alternative splice forms of the SCD4 gene, both of which are expressed in mouse skin. Alternatively, they may represent sequences from two SCD4 genes. These clones are unlikely to be cloning artifacts, since the difference was also noted at the 3' end just prior to the poly A stretch in the two sequences as shown in Figure 4 (boxed region of 6 nucleotides). The protein-coding regions are underlined, and are identical as between these two variants.

Comparisons of M-SCD 1-4 cDNA sequences

When comparing the cDNA sense strand sequences of all four mouse SCD genes, one sees that the regions of significant homology between SCD1 and SCD3 start at the 5' end of the non-coding region, whereas homology starts only within the coding sequences as between SCD2 and SCD4 (Figure 4). All species share varying degrees of homology in their protein-coding regions. The homology does not extend into the 3' non-coding region between SCD4 and SCD1 or SCD2

M-SCD3 protein sequence as deduced from cDNA sequence

The longest ORF of M-SCD3 cDNA that also has a high degree of homology to SCD1 protein-coding sequence is shown in Figure 5. Although this sequence lacks the

exon 6 sequence, it represents the sequence up to the end of exon 5, as indicated by homology to the M-SCD1 sequence. This identifies it as a bonafide member of the SCD family. M-SCD3 cannot be a splice variant of the SCD1 gene, since the differences in nucleotides that lead to a single amino acid change of alanine to cysteine at position 97 of the SCD3 protein sequence occur within an exon. In addition, SCD3 cDNA has a unique 3' non-coding sequence.

M-SCD4 protein sequence as deduced from cDNA sequence

The full protein sequence of 359 amino acids, as deduced from M-SCD4v1 cDNA, is shown in Figure 6. The sequence has all conserved histidine residues at the same locations as in the several species of desaturases and hyroxylases (Shanklin, et al. 1994).

Comparison of protein sequences of mouse SCD's

Figure 7 shows a comparison of mouse SCD1, SCD2, SCD3 and SCD4 protein sequences. SCD1 has an identical protein sequence to that of SCD3, except for one amino acid position where the alanine in SCD1 is replaced by cysteine in SCD3 (position 101 in Figure 7). SCD2 and SCD4 are more divergent and have more amino acid differences, which are not shared by SCD1 or SCD3. Those amino acids which differ between all four SCD's are underlined. The conserved histidine amino acid residues in mammals, yeast and other lower species are boxed at positions 120, 125, 157, 160, 161, 298, 301, and 302 in Figure 7. The positions and the neighboring amino acid residues of these histidine regions are

conserved in all four mouse protein sequences (SCD1-4) as shown in Figure 7.

Comparison of protein sequences of mouse SCD4 and published hamster FAR17-C.

Comparison of SCD4 and FAR17C protein sequences shows that they share about 91% identity. If one ignores those amino acid differences where the substitutions are from similar functional groups, then the similarity is about 96%. From this comparison of mouse SCD4 with the hamster protein, it can be concluded that SCD4 is most likely the mouse equivalent of the hamster protein.

In situ hybridization of M-SCD3 in mouse skin

A 129 bp fragment (Not1 digest of 5α5 plasmid) containing the sequence from the 5' UTR that is unique to M-SCD3 was cloned into the PBluescript KS vector (Stratagene), and was used for generating riboprobes. The M-SCD3-specific sequence is shown as boxed in Figure 1. The relationship of the M-SCD3-specific sequence (boxed) to that of M-SCD4v1 is shown in Figure 3. The dorsal skin of an adult C57Bl/6 mouse was excised and frozen in OCT embedding compound. Frozen sections were collected on positively-charged glass slides. To generate the antisense probe, M-SCD3-unique plasmid was first linearized with Sac1 and labeled with digoxigenin using T7 RNA polymerase according to manufacturer's instructions (Boehringer-Manheim). A sense, digoxigenin-labeled riboprobe was generated using T3 RNA polymerase with a BamH1-linearized plasmid.

In situ hybridization was carried out according to standard procedures (Hebert, et al. 1991). The results, not shown here, indicate that M-SCD3 is primarily expressed in matrix keratinocytes of the hair follicle and not in the sebaceous gland. The expression of M-SCD3 in matrix keratinocytes suggests an important role for M-SCD3 in either proliferation and/or differentiation of these cells. Cotsarelis, et al. (1990) have reported that, using tritiated thymidine, these cells are highly proliferative and comprise the transient-amplifying stem cells of the hair follicle.

It is most likely at this location in the hair follicle that critical decisions concerning growth are taking place. Thus, there are likely to be complex regulatory molecules and mechanisms functioning at this site, of which SCD may be one, especially in light of SCD up-regulation in proliferating cells (Diplock, et al. 1988). N-myc, a transcription factor involved in proliferation and differentiation, is expressed specifically in these cells (Mugrauer, et al. 1988). Growth factor receptors (FGFR2, Rosenquist, et al. 1996; IGFR, Hodak, et al. 1996) are expressed in these cells, suggesting a role in proliferation and/or differentiation.

The lack of expression in the sebaceous glands indicates that M-SCD3 may not function at this site. However, as will be discussed below, M-SCD4 may have a role in sebaceous function. Thus, it is concluded that, based on the expression pattern and putative function of SCD, the role of M-SCD3 in pilosebaceous function may be in regulation of hair follicle growth.

In situ hybridization of M-SCD4 in mouse skin

A 223 bp fragment (EcoR1-Sphl digest of clone 5a) containing the entire 5' UTR of M-SCD4v2 was cloned into the PBluescript SK vector (Stratagene). Position 130 to 225 of the M-SCD4v2 sequence is 100% identical to that of M-SCD4v1. Thus, this probe recognizes both variant forms of M-SCD4. Mouse skin for ISH was prepared as described above. An anti-sense, digoxigenin-labeled riboprobe was generated using T7 RNA polymerase with a EcoR1-linearized plasmid. A sense, digoxigenin-labeled riboprobe was generated using T3 RNA polymerase with a Kpnl-digested plasmid. ISH was carried out as described above.

The results, not shown here, show that in full thickness skin, M-SCD4 is strongly expressed in the matrix keratinocytes of the hair follicle. Sebaceous glands express M-SCD4. The follicular papilla ("FP") of hair follicles and epidermis do not express M-SCD4. Adjacent follicular papilla fibroblasts are negative for M-SCD4 mRNA. Sebaceous glands specifically express M-SCD4 in the lower aspect of the gland, although some sebaceous glands do not express significant amounts of M-SCD4. The reasons for this are not clear at present, but may be related to heterogeneity of M-SCD4-expressing cells within the gland, cyclical expression in glands, or plane of section of the tissue sample.

Based on the expression pattern and putative function of SCD, M-SCD4 apparently plays a role in both hair follicle growth and sebaceous gland function. That M-SCD4, and not M-SCD3, is expressed in sebaceous

glands is consistent with the expression of FAR17c, which is the hamster homolog of M-SCD4. FAR17c is more related to M-SCD4 than to M-SCD3 in cDNA and protein sequence. FAR17c was isolated from hamster flank organ, a tissue comprised mostly of sebaceous cells. No localization data are presented in the work on FAR17c, thus this localization of M-SCD4 to sebaceous glands represents the first anatomical localization of SCD to vertebrate sebaceous glands.

The presence of both M-SCD4 and M-SCD3 in the matrix keratinocytes suggests that redundancy may be related to a critical role for this enzyme in matrix cell physiology. It is notable that no expression is seen in the epidermis, a site of putative epidermal stem cells, suggesting that M-SCD3 and 4 may have a function specific to hair follicle (and possible sebaceous gland) stem cells.

cDNA sequence of human SCD

Using primers based on a partial cDNA sequence (in database Wisconsin Package Version 9.1, Genetics Computer Group, GCG, Madison, Wisconsin) from adipose tissue and a cDNA sequence from M-SCD1, PCR was used to amplify the complete open reading frame (ORF), as well as to generate probes, from cDNA of human scalp PSU's. These probes were used to screen a human foreskin keratinocyte cDNA library, from which the 5' untranslated region (UTR), the complete ORF, and part of the 3' UTR were cloned. Expression of HS-SCD is present in the matrix keratinocytes of the hair follicle and in the sebaceous gland. This pattern is similar to that in mouse, and suggests the conservation

of an important function in skin. Additionally, HS-SCD is expressed in eccrine sweat glands. It is important to note that unlike humans, mice do not have eccrine sweat glands in their hair-bearing skin.

Total RNA was isolated from human scalp "hair plugs" (the complete pilosebaceous unit) using RNA-STAT according to manufacturer's instructions (Tel Test, Inc). First strand cDNA synthesis was performed using the Advantage RT-for-PCR kit according to manufacturer's instructions (Clontech #K1402-1). A pair of primers (forward: 5'GATATCTCAAGCTCCTATACC3', reverse: 5'CTCCTCTGGAACATCACCAGTT3'), corresponding to positions 20-40 and 621-642, respectively, of HA-SCD (Figure 10), was used to amplify skin SCD sequence by PCR. The PCR fragment was cloned into the PBluescript vector and then used to generate a cDNA probe to screen a human foreskin keratinocyte (HFKC) cDNA lambda library constructed in λgt11 (Clontech #HL1110B).

From the HFKC cDNA library, 8 overlapping clones were generated that comprised the 5' UTR, the complete ORF, and a portion of the 3' UTR (Figure 8). Using additional primers based on M-SCD1 and HL-SCD, the complete ORF, as well as portions of the 5' and 3' UTR, were generated by PCR from hair plug cDNA. This sequence was compared with that obtained from the HFKC cDNA library.

All sequences were identical with exception of the codon for amino acid 224. cDNA sequences from the HFKC library indicate a C at position 898 (see Figure 8), which results in the amino acid leucine. cDNA sequences of "uncloned" PCR products from the hair

plugs indicate both a C and A at position 898, indicating that individual SCD transcripts from hair plug have either a C (producing leucine at amino acid 224) or an A (producing methionine at amino acid 224) at position 898 of the cDNA sequence. In Figures 8-11, all HS-SCD sequences are reported as C at position 898 of cDNA. The amino acid sequence in Figure 11 is reported with leucine at position 224. Since the hair plug samples are pooled from several individuals, the changes seen at bp 898 may be due to polymorphism.

The ORF is 1080 bp and generates a predicted protein of 359 amino acids. Included in the human skin cDNA is 228 bp of 5' UTR and 689 bp of 3' UTR.

Comparison of human skin SCD with human liver SCD

The cDNA sequences of human skin and liver SCD are 97.9% identical at the nucleotide level and 98.3% identical at the protein level. A total of 30 nucleotide differences exist between the two cDNA sequences (Figure 9), and a total of 6 amino acid differences exist in the protein sequence (Figure 11).

Of the nucleotide differences, 3 occur in the 5' UTR, 8 in the ORF, and 17 in the 3' UTR (Figure 9). Of the eight differences in the ORF, 6 lead to amino acid changes. Of these 6 changes, 5 of the 6 base substitutions occur either at the first or second position of the codon. The base substitutions in the ORF of liver compared to skin are as follows (using skin sequence as reference): bp 300: T to C, bp 301: C to T, bp 303: A to C, bp 304: G to A, bp 898: A to C,

bp 1187: A to C, bp 1206: G to C, and bp 1225: A to G (Figure 9).

The most significant base transitions are bp 301 and bp 304. The substitution at these sites in liver vs skin result in amino acid substitutions of proline to serine (amino acid 25) and in glycine to arginine (amino acid 26), respectively (Figure 11). The substitution of serine for proline in the skin cDNA may increase the polarity and may alter the secondary structure conferred by proline. The dramatic substitution of arginine for glycine in the skin cDNA replaces a nonpolar amino acid with a postively charged amino acid, which may result in an altered surface profile. The substitution of G for A at bp 1225 (corresponding to amino acid 333) in the skin cDNA results in the replacement of threonine with alanine, a residue change from polar to nonpolar which may also affect the surface profile (Figure 11). The substitution of C for G at bp 1206 in HS-SCD replaces tryptophan with cysteine at amino acid 326. This replaces an aromatic ring with a thiol group, which can confer different secondary structure through a disulfide bond.

The remaining substitutions (in skin cDNA from liver cDNA) at bp 898 and 1187 result in the replacement of amino acid residues of a similar biochemical profile. These replacements are: methionine to leucine (AA 224, nonpolar), and asparagine to threonine (AA 320, polar) (Figure 11).

The 5' UTR contains 20 bp of unique sequence not present in the liver cDNA. The 3' UTR contains an

additional 508 bp not contained in the liver cDNA. The significance of the base pair differences in the UTR's is unknown. However, these differences may result in altered stability of the mRNA. The 17 differences in the 3' UTR are mostly associated with the stretch of A's present in the liver cDNA.

Comparison of human skin SCD with human adipose SCD

The human adipose SCD represents a partial cDNA that is completely contained within the ORF of HS-SCD and HL-SCD. The skin cDNA sequence that overlaps the adipose cDNA sequence is 97.8% identical. The overlapping protein-coding sequence is 97.4% identical.

The cDNA sequence of skin contains 15 base changes from the adipose cDNA sequence as seen in Figure 10. Of the 15 differences in the ORF, 6 lead to amino acid changes. Of these 6 changes, 5 of 6 base substitutions occur either at the first or second position of the codon. The base substitutions in the ORF of adipose compared to skin are as follows (using skin as the reference): bp 241: A to T, bp 246: C to G, bp 249: A to G, bp 252: G to C, bp 261: A to T, bp 300: T to C, bp 301: C to T, bp 303: A to C, bp 304: G to A, bp 393: C to T, bp 888: C to T, bp 898: A to C, bp 936: C to T, bp 938: G to T, and bp 945: C to T (Figure 10).

The most dramatic substitutions are in bp 301 and 304. The substitution at 301 results in proline to serine, and the substitution at 304 results in glycine to arginine (Figure 10). These are the same changes in amino acids 25 and 26 that are seen in HL-SCD cDNA as compared to HS-SCD cDNA. The substitution of T for G

at bp 938 of HS-SCD results in replacement of cysteine with phenylalanine at amino acid 237, which may change secondary structure via altered disulfide bridges. The substitions (in skin cDNA from adipose cDNA) at bp 241, 252, and 898 result in the replacement of amino acid residues of similar biochemical profile. These replacements are: methionine to leucine (nonpolar), glutamate to aspartate (acidic), and methionine to leucine (nonpolar), respectively. The base changes at 246, 249, 261, 393, 888, 936, and 945 do not result in amino acid changes.

Comparison of human skin SCD with MLD

The comparison of human skin SCD with MLD results in 36.7% identity at the nucleotide level, and no identity at the amino acid level. This indicates that the MLD is a highly divergent human SCD family member. MLD does contain the conserved histidine regions that are common to the SCD family.

The sequence comparisons described in the above sections indicate that HS-SCD is a highly related, but unique sequence from HL-SCD and HA-SCD. HS-SCD contains a serine and arginine at amino acids 24 and 25, where both HL-SCD and HA-SCD contain proline and glycine at these positions. These particular substitutions, resulting in substitutions of adjacent amino acids, are corroborated by the same serine and arginine found in the porcine SCD. No substitution between skin, liver, and adipose SCD disrupts or occurs within the conserved histidine motifs as indicated by underlining in Figure 11. The functional significance of these substitutions are presently unknown. However, it has been

demonstrated that a single amino acid substitution can alter substrate specificity of p450 enzymes (Lindberg, et al. 1989), the super-family to which SCD belongs.

Expression of HS-SCD in pilosebaceous unit and eccrine sweat glands

The ISH probe used for localization of HS-SCD in skin is the same as that used to screen the HFKC cDNA library, as described above, and is shown boxed in Figure 8. This region is highly homologous to the corresponding regions of liver SCD and adipose SCD, and thus would be expected to cross react in any procedure utlizing hybridization. However, at no time could liver or adipose SCD sequences be detected in hair plug cDNA or in the HFKC library when sequencing both cloned and un-cloned PCR products. Nevertheless, a possible polymorphism was detected at bp 898. No other base positions were called ambiguously (an N in the base sequence, indicating "any" base). Since the liver and adipose sequences differ from the skin SCD sequence by many base pairs, one would expect to see "N" called at these positions when sequencing un-cloned PCR products, if in fact the liver and adipose transcripts were expressed in skin. Since this result was never seen, it can be concluded that skin only expresses the skin SCD sequence as given in Figure 8. Thus the ISH probe, although based on a common region of cDNA, should only detect the skin SCD on tissue sections of hair plug samples.

The matrix keratinocytes of the hair bulb strongly and specifically expresses HS-SCD as indicated by data not shown here. Adjacent FP fibroblasts do not express

HS-SCD. This expression pattern is strikingly similar to that of mouse. Although expressed less prominently than in the matrix cells, HS-SCD is specifically expressed in the human sebaceous gland.

A function similar to that of mouse SCD in sebaceous gland could be proposed. Eccrine sweat glands specifically express HS-SCD. The presence of HS-SCD in eccrine sweat gland suggests that HS-SCD may function in the growth regulation of the eccrine sweat gland cells and/or in modification of the lipid contained in sweat.

Expression of H-SCD in hair matrix keratinocytes

Experiments were performed that show that SCD mRNA is highly expressed in the hair matrix keratinocytes of both mouse and human, suggesting a phylogenetically conserved function. Cell division is highly conserved throughout evolution. Increased SCD has been found in several human tumors (Li et al., 1994), and is up-regulated in cells that are placed in culture and undergoing rapid growth as determined here by experiment. Similarly, it is down-regulated in cells that have stopped dividing, as determined here by experiment.

In light of this, it becomes possible to treat hypertrichosis and hirsutism by down-regulating SCD to stop or slow proliferation, and thereby prevent hair growth. On the other hand, it also becomes possible to initiate or augment hair growth by up-regulating SCD to increase proliferation in the hair matrix cells and thereby treat alopecia.

References

Agrawal, S. (1996) Antisense Theraputics, Totowa, NJ, Humana Press, Inc.

Ahmad, W., et al. (1998) Science 279(5351):720-4.

Ailhaud, G., et al. (1995) Prostaglandins Leukot Essent Fatty Acids 52(2-3):113-5.

Alessi, D.R., et al. (1998) Curr Opin Genet Dev 8(1):55-62.

Barry, Ed. (1983) Formulation of Dermatological Vehicles, pp. 296-350; Marcel Dekker, Inc., New York and Basel.

Berry, E.M. (1997) Handbook of essential fatty acid biology: biochemistry, physiology, and behavioral neurobiology, Yehuda, S. and Mostofsky, D.I., Eds.; Totowa, NJ, Humana Press, Inc.; pp. 89-101.

Bocos, C., et al. (1995) J Steroid Biochem Mol Biol 53(1-6):467-73.

Cadena, D.L., et al. (1997) Biochemistry 36(23):6960-7.

Casimir, D.A., et al. (1996) J Biol Chem 271(47):29847-53.

Chen, Y.L., et al. (1996) J Invest Dermatol 107(1):15-9.

Christy, R.J., et al. (1989) Genes Dev 3(9):1323-35.

Clandinin, M.T., et al. (1991) FASEB J 5(13):2761-9.

Cotsarelis, et al. (1990) Cell 61(7):1329-1337.

Diaz-Guerra, M.J., et al. (1991) J Biol Chem 266(35):23568-76.

Diplock, et al. (1988) Biochim Biophys Acta 962(1)42-50.

Dlugosz, A.A., et al. (1993) J Cell Biol 120(1):217-25.

Downing, D.T., et al. (1986) J Am Acad Dermatol 14(2 Pt 1):221-5.

Enoch, H.G., et al. (1976) J Biol Chem 251(16):5095-103.

Eramo, L.R., et al. (1985) Arch Dermatol 121(9):1167-74.

Farrell, Jr., R.E. (1993) RNA Methodologies: A Laboratory Guide for Isolation and Characterization, San Diego, CA, Academic Press, Inc.

Fowler, K.J., et al. (1995) Proc Natl Acad Sci (USA) 92(5):1465-9.

Gates, A.H., et al. (1965) Science 148:1471-3.

Gyorfi, et al. (1997) Biochim Biophys Res Commun 241(2)465-470.

Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual; Cold Spring Harbor, NY, Cold Spring Harbor Laboratory.

Harmon, C.S., et al. (1995) Br J Dermatol 133(5):686-93.

Hebert, J.M., et al. (1994) Cell 78(6):1017-25.

Henderson, R.J., et al. (1992) Lipid Analysis: A Practical Approach, Hamilton, R.J., and Hamilton, S., Eds.; New York and Tokyo, Oxford University Press; pp.65-111.

Hodak, et al. (1996) J Invest Dermatol 106(3):564-570.

Hogan, B., et al. (1986) Manipulating the Mouse Embryo. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory.

Holman, R.T. (1993) Essential fatty acids and eicosanoids, Sinclair, A. and Gibson, R., Eds.; Champaign, IL, American Oil Chemists Society; pp. 3-17.

Hrelia, S., et al. (1994) Biochem Mol Biol Int 34(3):449-55.

Hughes, Jr., C.L. (1994) Hirsutism, in Disorders of Hair Growth: Diagnosis and Treatment. Olsen, E.A., Ed., New York, NY, pp. 337-351.

Jones, L.N., et al. (1996) J Invest Dermatol 106(3):461-4.

Jong, M.C., et al. (1998) J Clin Invest 101(1):145-52.

Josefowicz, W.J., et al. (1978a) Genet Res 31(1):53-65.

Josefowicz, W.J., et al. (1978b) Genet Res 31:145-51.

Josefowicz, W.J., et al. (1978c) Genet Res 31:157-66.

Kaestner, K.H., et al. (1989) J Biol Chem 264(25):14755-61.

Kasai, R., et al. (1976) Biochemistry 15(24):5228-33.

Kates, M., et al. (1984) Membrane fluidity, Kates, M. and Manson, L.A., Eds.; New York and London, Plenum Press; pp. 379-95.

Koch, P.J., et al. (1997) J Cell Biol 137(5):1091-102.

Li, J., et al. (1994) Int J Cancer 57(3):348-52.

Lindberg and Nigishi (1989) Nature 339(6226):632-634.

Lippiello, P.M., et al. (1979) J Biol Chem 254(6):2004-9.

Liscovitch M, et al. (1994) Cell 77(3):329-34.

Mann, G.B., et al. (1993) Cell 73(2):249-61.

Martindale's Extra Phamacopoeia, 31st Ed. (1996).

Miettinen, P.J., et al. (1995) Nature 376(6538):337-41.

Mihara, K. (1990) J Biochem (Tokyo) 108(6):1022-9.

Miller, C.W., et al. (1996) Proc Natl Acad Sci (USA) 93(18):9443-8.

Mugrauer, et al. (1988) J Cell Biol 107(4)1325-1335.

Neely, J.R., et al. (1974) Annu Rev Physiol 36:413-59.

Nehls, M., et al. (1994) Nature 372(6501):103-7.

Ntambi, J.M. (1995) Prog Lipid Res 34(2):139-50.

Ntambi, J.M., et al. (1988) J Biol Chem 263(33):17291-300.

Olsen, E.A. (1994) Hypertrichosis, in Disorders of Hair Growth: Diagnosis and Treatment; Olsen, E.A., Ed., New York, NY, pp. 315-336.

Orr-Urtreger A., et al. (1992) Development 115(4):1045-58.

Oshino, N. (1972) Arch Biochem Biophys 149(2):378-87.

Oshino, N., et al. (1972) Arch Biochem Biophys 149(2):369-77.

Ozols, J. (1997) Mol Biol Cell 8(11):2281-90.

Pennycuik, P.R., et al. (1986) Genet Res 48(3):179-85.

Ramchandani, M. and Toddywala, R. (1997) Formulation of Topical Delivery Systems, pp. 539-578; Eds. Ghosh and Pfister; Interpharm Press, Inc., Buffalo Grove, Illinois.

Rasmussen, H.H., et al. (1993) J Invest Dermatol 101(4):560-6.

Rietschel, R.L. (1996) Dermatological Clinics 14 (4): 691-695.

Rosenquist, et al. (1996) Dev Dyn 205(4):379-386.

Rosseneu, M., et al. (1995) FASEB J 9(9):768-76.

Sambrook, J., et al. (1989) Molecular Cloning: A Laboratory Manual; Cold Spring Harbor, NY, Cold Spring Harbor Laboratory.

Shanklin, J. et al. (1994) Biochemistry 33(43):12787-94.

Shinomura T., et al. (1991) Proc Natl Acad Sci (USA) 88(12):5149-53.

St. John, L.C., et al. (1991) J Anim Sci 69(3):1064-73.

Strittmatter, P., et al. (1974) Proc Natl Acad Sci (USA) 71(11):4565-9.

Sunberg, J.P. (1994) Handbook of mouse mutations with skin and hair abnormalities: animal models and biomedical tools, Sundberg, J.P., Ed.; Bar Harbor, MA, The Jackson Laboratory; pp. 9-19.

Tiku, P.E., et al. (1996) Science 271(5250):815-8.

Toker, A., et al. (1997) Nature 387(6634):673-6.

U.S. Pharmacopeia, XXIII (1995).

Wahle, E.W., et al. (1975) Br J Nutr 34(1):105-12.

Wenniger, et al. (1997) International Cosmetic Ingredient Dictionary and Handbook, 7th Ed., Vol 1-3.

Wevers, A., et al. (1992) Arch Dermatol Res 284(1):5-7.

Wilkinson, D.I., et al. (1966) J Invest Dermatol 47(5):449-55.

Williams, D., et al. (1994) Dev Biol 165(2):469-79.

Youn, S.P., et al. (1998) J Invest Dermatol 110:680.

What is claimed is:

1. A nucleic acid molecule encoding the human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof.

2. The nucleic acid molecule of claim 1, wherein the molecule is a DNA molecule.

3. The DNA molecule of claim 2, wherein the molecule is a cDNA molecule.

4. The cDNA molecule of claim 3 comprising the sequence shown in Figure 8.

5. The DNA molecule of claim 2, wherein the molecule is in an expression vector.

6. A nucleic acid molecule which, under suitable conditions, specifically hybridizes to a nucleic acid molecule encoding the human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof.

7. The nucleic acid molecule of claim 6, wherein the molecule is labeled with a detectable marker.

8. A method of diagnosing a human subject for a skin disorder characterized by an abnormal level of stearoyl-CoA desaturase expression, which comprises (i) obtaining a sample of skin mRNA from the subject; (ii) contacting the sample so

obtained with an excess of the labeled nucleic acid molecule of claim 7 under conditions permitting hybridization of the labeled nucleic acid molecule with stearoyl-CoA desaturase mRNA present in the sample; (iii) removing un-hybridized labeled nucleic acid molecule from the sample; (iv) quantitatively determining the amount of hybridized labeled nucleic acid molecule present in the sample; and (v) comparing the amount determined in step (iv) with the amount determined using a skin mRNA sample from a normal human subject, a difference in these amounts being correlative of an abnormal level of stearoyl-CoA desaturase expression in the skin of the subject being diagnosed.

9. An isolated human stearoyl-CoA desaturase encoded by the nucleic acid molecule of claim 1.

10. The isolated desaturase of claim 9 having the amino acid sequence shown in Figure 8.

11. A eukaryotic cell line which expresses human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof, wherein the cell line is transfected with an expression vector encoding the desaturase.

12. The eukaryotic cell of claim 11, wherein the cell is a mammalian cell.

13. A method for determining whether an agent increases the expression level of human stearoyl-CoA desaturase in skin cells already expressing

same, which comprises the steps of (i) contacting the agent under suitable conditions with a eukaryotic cell line expressing human stearoyl-CoA desaturase at a known level; and (ii) determining whether the stearoyl-CoA desaturase expression level increases after cellular contact with the agent, thereby determining whether the agent increases the expression level of human stearoyl-CoA desaturase in skin cells already expressing same.

14. A method for determining whether an agent decreases the expression level of human stearoyl-CoA desaturase in skin cells already expressing same, which comprises the steps of (i) contacting the agent under suitable conditions with a eukaryotic cell line expressing human stearoyl-CoA desaturase at a known level; and (ii) determining whether the stearoyl-CoA desaturase expression level decreases after cellular contact with the agent, thereby determining whether the agent decreases the expression level of human stearoyl-CoA desaturase in skin cells already expressing same.

15. The method of claim 13 or 14, wherein the eukaryotic cell line is a cell line transfected with an expression vector encoding a human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof.

16. The method of claim 13 or 14, wherein the eukaryotic cell line is a non-transfected cell line.

17. A method for determining whether an agent decreases the activity of human stearoyl-CoA desaturase in skin cells, which comprises the steps of (i) contacting the agent under suitable conditions with human stearoyl-CoA desaturase having a known level of activity; and (ii) determining whether the desaturase activity decreases after contact with the agent, thereby determining whether the agent decreases human stearoyl-CoA desaturase activity in skin cells.

18. A method for determining whether an agent increases the activity of human stearoyl-CoA desaturase in skin cells, which comprises the steps of (i) contacting the agent under suitable conditions with human stearoyl-CoA desaturase having a known level of activity; and (ii) determining whether the desaturase activity increases after contact with the agent, thereby determining whether the agent increases human stearoyl-CoA desaturase activity in skin cells.

19. An antibody which specifically binds to human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof, and thereby inhibits the activity thereof.

20. A pharmaceutical composition for treating a human skin disorder characterized by an excess of

stearoyl-CoA desaturase activity, which comprises the antibody of claim 19 and a pharmaceutically acceptable carrier for use in topical administration.

21. An expression vector suitable for use in gene therapy, which vector encodes a nucleic acid molecule capable of specifically inhibiting the expression of human skin stearoyl-CoA desaturase.

22. The expression vector of claim 21, wherein the nucleic acid molecule is an anti-sense molecule which is complementary to, and specifically hybridizes with, at least a portion of human stearoyl-CoA desaturase mRNA.

23. A pharmaceutical composition for treating a human skin disorder characterized by an excess of skin stearoyl-CoA desaturase activity, which comprises the expression vector of claim 21, and a pharmaceutically acceptable carrier for use in topical administration.

24. A method for treating a human subject afflicted with a skin disorder characterized by an excess of stearoyl-CoA desaturase activity, which comprises topically administering to the subject a therapeutically effective dose of the pharmaceutical composition of claim 20 or 23.

25. The method of claim 24, wherein the disorder is selected from the group consisting of skin cancer, hypertrichosis and hirsutism.

26. The DNA molecule of claim 5, wherein the expression vector is suitable for use in gene therapy.

27. A pharmaceutical composition for treating a human skin disorder characterized by insufficient skin stearoyl-CoA desaturase activity, which comprises the expression vector of claim 26, and a pharmaceutically acceptable carrier for use in topical administration.

28. A method for treating a human subject afflicted with a skin disorder characterized by insufficient stearoyl-CoA desaturase activity, which comprises topically administering to the subject a therapeutically effective dose of the pharmaceutical composition of claim 27.

29. The method of claim 28, wherein the disorder is selected from the group consisting of acne, atopic dermatitis and alopecia.

30. The antibody of claim 19, wherein the antibody is labeled with a detectable marker.

31. An antigen suitable for use in generating the antibody of claim 19, which comprises at least a portion of human stearoyl-CoA desaturase.

32. A method of producing the antibody of claim 19, which comprises the steps of administering to a suitable animal an antigenic portion of human stearoyl-CoA desaturase, and after a suitable length of time, isolating the antibody generated

by the animal against the antigenic portion so administered.

33. A method of diagnosing a human subject for a skin disorder characterized by an abnormal level of stearoyl-CoA desaturase expression, which comprises (i) obtaining a stearoyl-CoA desaturase-containing sample from the subject's skin; (ii) contacting the sample so obtained with an excess of the antibody of claim 19 under conditions permitting binding of the antibody with stearoyl-CoA desaturase present in the sample; (iii) removing un-bound antibody from the sample; (iv) quantitatively determining the amount of bound antibody present in the sample; and (v) comparing the amount determined in step (iv) with the amount determined using a skin stearoyl-CoA desaturase sample from a normal human subject, a difference in these amounts being correlative of an abnormal level of stearoyl-CoA desaturase expression in the skin of the subject being diagnosed.

34. A transgenic mouse whose skin cells do not express any gene encoding mouse skin stearoyl-CoA desaturase having the amino acid sequence shown in Figure 1 or 2, or any polymorphism thereof.

35. The transgenic mouse of claim 34, wherein the mouse has operably integrated into its chromosomes a DNA sequence encoding human stearoyl-CoA desaturase having the amino acid sequence shown in Figure 8 or a polymorphism thereof, which desaturase is expressed in the mouse's skin cells.

```
   1  CCTCGCCCAC GGCACCCACA GTGCCCCACA TGCCGGCTGT GGGAATCCAG
  51  TTTCTGGCCA GTTCCCGGTA GCGCGGGCCT AGAAACCTGC TCAGCATCGC
 101  TGCAGGGTCT CAATACTCAG GAATTCAGCG GCCGCCCGGG CAGGTGCTGA
 151  ACACCCATCC CGAGAGTCAG GAGGGCAGGT TTCCAAGCGC AGTTCCGCCA
 201  CTCGCCTACA CCAACGGGCT CCGGAACCGA AGTCCACGCT CGATCTCAGC
 251  ACTGGGAAAG TGAGGCGAGC AACTGACTAT CATCATGCCG GCCCACATGC
 301  TCCAAGAGAT CTCCAGTTCT TACACGACCA CCACCACCAT CACTGCACCT
 351  CCCTCCGGAA ATGAACGAGA GAAGGTGAAG ACGGTGCCCC TCCACCTGGA
 401  AGAAGACATC CGTCCTGAAA TGAAAGAAGA TATTCACGAC CCCACCTATC
 451  AGGATGAGGA GGGACCCCCG CCCAAGCTGG AGTACGTCTG GAGGAACATC
 501  ATTCTCATGG TCCTGCTGCA CTTGGGAGGC CTGTACGGGA TCATACTGGT
 551  TCCCTCCTGC AAGCTCTACA CCTGCCTCTT CGGGATTTTC TACTACATGA
 601  CAAGCGCTCT GGGCATCACA GCCGGGGCTC ATCGCCTCTG GAGCCACAGA
 651  ACTTACAAGG CACGGCTGCC CCTGCGAATC ITCCTTATCA TTGCCAACAC
 701  CATGGCGTTC CAGAATGACG TGTACGAATG GGCCCGAGAT CACCGCGCCC
 751  ACCACAAGTT CTCAGAAACA CACGCCGACC CTCACAATTC CCGCCGTGGC
 801  TTCTTCTTCT CTCACGTGGG TTGGCTGCTT GTGCGCAAAC ACCCGGCTGT
 851  CAAAGAGAAG GGCGGAAAAC TGGACATGTC TGACCTGAAA GCCGAGAAGC
 901  TGGTGATGTT CCAGAGGAGG TACTACAAGC CCGGCCTCCT GCTGATGTGC
 951  TTCATCCTGC CCACGCTGGT GCCCTGGTAC TGCTGGGGCG AGACTTTTGT
1001  AAACAGCCTG TTCGTTAGCA CCTTCTTGCG ATACACTCTG GTGCTCAACG
1051  CCACCTGGCT GGTGAACAGT GCCGCGCATC TCTATGGATA TCGCCCCTAC
1101  GACAAGAACA TTCAATCCCG GGAGAATATC CTGGTTTCCC TGGGTGCCGT
1151  GG
```

Fig. 2 **Mouse Skin SCD4v1 cDNA Sequence.**

```
   1  CCCAAGCTGA CTCTGGGCTT CTGTGAGTGC TCCTGAAGGC TGAAGTTCTG
  51  TGGTGGCATC GAGGGCCCAC TGAGCATGGG TCCTGGGCTT AGCTCTTCTC
 101  AACTGCTGTC CTCAGCTTAA AAGGGGATAA ATGAAACCAA CTCTCTGCTG
 151  CTTTAGCAGA GGACATGGAG AAACCCGGAC CCCACGATCA CATCTGGACC
 201  AGAGAGTATT GCAAATCCAG AAAACAGGAT CTGCAACAGA AGCCTCCTCT
 251  GCCCTGCAGC CCCAAACGCC ACAACTTTAA ATCCTTGGAA GATCTTCCCG
 301  GCCTCCAAGA ACCAGCATGC CAGGGCACCT GCTGCAAGAA GAGATGACGC
 351  CTTCGTACAC GACCACCACC ACCATCACAG CGCCTCCCTC TGGAAGCCTG
 401  CAGAATGGAC GAGAGAAGGT GAAGACGGTG CCCCTCTACC TGGAAGAAGA
 451  CATCCGTCGT GAAATGAAAG AAGATATATA CGACCCCACC TATCAGGATG
 501  AGGAGGGGCC CCCGCCCAAG CTGGAGTACG TCTGGAGGAA CATCATTCTC
 551  ATGGCCCTGC TGCACGTGGG AGCCCTGTAC GGGATCACAC TGGTTCCCTC
 601  CTGCAAGCTC TACACCTGCC TCTTCGCGTT TGTCTACTAT GTGATCAGTA
 651  TTGAGGGCAT TGGAGCCGGA GTCCATCGCC TGTGGAGCCA CAGAACGTAC
 701  AAGGCACGCC TGCCCCTGCG GATCTTCCTC ATCATTGCCA ACACCATGGC
 751  GTTCCAGAAT GACGTGTATG AATGGGCCCG AGATCACCGA GCCCACCACA
 801  AGTTCTCAGA AACACACGCC GACCCTCACA ATTCCCGCCG TGGCTTCTTC
 851  TTCTCTCACG TGGGTTGGCT GCTTGTGCGC AAACACCCGG CTGTCAAAGA
 901  GAAGGGCGGA AAACTGGACA TGTCTGACCT GAAAGCCGAG AAGCTGGTGA
 951  TGTTCCAGAG GAGGTACTAC AAGCCTGGCA TTCTGCTGAT GTGCTTCATC
1001  CTGCCCACGC TGGTGCCCTG GTACTGCTGG GGCGAGACTT TTCTAAACAG
1051  TTTTTATGTT GCCACTTTAC TGAGATACGC TGTGGTGCTC AACGCCACTT
1101  GGCTGGTGAA CAGTGCCGCC CACCTCTACG GGTATCGCCC CTACGATAAG
1151  AACATCGATC CCGGGCAGAA TGCCCTGGTT TCCTTGGGAA GTATGGGCGA
```

Fig. 2 (cont

1201 GGGCTTCCAC AACTACCACC ATGCCTTCCC CTACGACTAC TCTGCCAGTG

1251 AGTACCGCTG GCACATCAAC TTCACCACGT TCTTCATCGA CTGCATGGCT

1301 GCACTGGGCC TGGCTTACGA CCGGAAGAGA GTGTCCAAGG CCACTGTCTT

1351 AGCCAGGATT AAGAGAACTG GAGACGGGAG TCACAAGAGT GGCTGAATTT

1401 GGAGTCAGTC TATTCCAAAA GCCAGCTGGA TAGGGGTTTA ATAATGTTTT

1451 TTCAAATACC GAAAAGAAGC ACCCATGTTG TATAGTGTCC TACTTCAAGA

1501 CAATATTCTT GTAAAATATT CAAATATTAA AAGACCAAAA CTTTCTTTTA

1551 TGATGCTAAA AAAAAAAAAA AAAAA

Fig. 3 **Homology between Mouse Skin SCD4v1 and SCD3 cDNA Sequences.**

Fig. 3 (cont.)

```
507 ATGGTCCTGCTGCACTTGGGAGGCCTGTACGGGATCATACTGGTTCCCTC 556
    |||| |||||||||| |||||| ||||| |||||||||| ||||||||||
551 ATGGCCCTGCTGCACGTGGGAGCCCTGTACGGGATCACACTGGTTCCCTC 600

557 CTGCAAGCTCTACACCTGCCTCTTCGGGATTTTCTACTACATGACAAGCG 606
    |||||||||||||||||||||||| | || ||||||| ||| ||
601 CTGCAAGCTCTACACCTGCCTCTTCGCGTTTGTCTACTATGTGATCAGTA 650

607 CTCTGGGCATCACAGCCGGGGCTCATCGCCTCTGGAGCCACAGAACTTAC 656
     | |||||| |||||| | |||||||| |||||||||||||| |||
651 TTGAGGGCATTGGAGCCGGAGTCCATCGCCTGTGGAGCCACAGAACGTAC 700

657 AAGGCACGGCTGCCCCTGCGAATCTTCCTTATCATTGCCAACACCATGGC 706
    |||||||| |||||||||| |||||||| |||||||||||||||||||
701 AAGGCACGCCTGCCCCTGCGGATCTTCCTCATCATTGCCAACACCATGGC 750

707 GTTCCAGAATGACGTGTACGAATGGGCCCGAGATCACCGCGCCCACCACA 756
    |||||||||||||||| |||||||||||||||||| ||||||||||
751 GTTCCAGAATGACGTGTATGAATGGGCCCGAGATCACCGAGCCCACCACA 800

757 AGTTCTCAGAAACACACGCCGACCCTCACAATTCCCGCCGTGGCTTCTTC 806
    ||||||||||||||||||||||||||||||||||||||||||||||||
801 AGTTCTCAGAAACACACGCCGACCCTCACAATTCCCGCCGTGGCTTCTTC 850

807 TTCTCTCACGTGGGTTGGCTGCTTGTGCGCAAACACCCGGCTGTCAAAGA 856
    |||||||||||| |||||||||||||||||||||| ||||||||||||
851 TTCTCTCACGTGGGTTGGCTGCTTGTGCGCAAACACCCGGCTGTCAAAGA 900

857 GAAGGGCGGAAAACTGGACATGTCTGACCTGAAAGCCGAGAAGCTGGTGA 906
    ||||||||||||||||||||||||||||||||||||||||||||||||
901 GAAGGGCGGAAAACTGGACATGTCTGACCTGAAAGCCGAGAAGCTGGTGA 950

907 TGTTCCAGAGGAGGTACTACAAGCCCGGCCTCCTGCTGATGTGCTTCATC 956
    |||||||||||||||||||||||| ||| | ||||||||||||||||||
951 TGTTCCAGAGGAGGTACTACAAGCCTGGCATTCTGCTGATGTGCTTCATC 1000

957 CTGCCCACGCTGGTGCCCTGGTACTGCTGGGGCGAGACTTTTGTAAACAG 1006
    |||||||||||||||||||||||||||||||||||| |||| |||||||
1001 CTGCCCACGCTGGTGCCCTGGTACTGCTGGGGCGAGACTTTTCTAAACAG 1050

1007 CCTGTTCGTTAGCACCTTCTTGCGATACACTCTGGTGCTCAACGCCACCT 1056
     | |  |||  ||| ||  ||  ||||| ||  ||||||||||||||| |
1051 TTTTTATGTTGCCACTTTACTGAGATACGCTGTGGTGCTCAACGCCACTT 1100

1057 GGCTGGTGAACAGTGCCGCGCATCTCTATGGATATCGCCCCTACGACAAG 1106
     |||||||||||||||||| || ||||| || |||||||||| |||
1101 GGCTGGTGAACAGTGCCGCCCACCTCTACGGGTATCGCCCCTACGATAAG 1150

1107 AACATTCAATCCCGGGAGAATATCCTGGTTTCCCTGGGTGCCGTGG.... 1152
     |||||  |  ||||| ||||| |||||||||| ||||     |||
1151 AACATCGATCCCCGGCAGAATGCCCTGGTTTCCTTGGGAAGTATGGGCGA 1200
```

Fig. 4 Comparison of the four Mouse SCD NA Sequences.

```
      1                                                  50
SCD1  .......... .......... .......... .......... ..........
SCD3  .......... .......... .......... ....CCTCGC CCACGGCACC
SCD2  .......... .........A TTCTGACTCC TGGACACCGG TGGCTGCAAG
SCD4  CCCAAGCTGA CTCTGGGCTT CTGTGAGTGC TCCTGAAGGC TGAAGTTCTG

      51                                                 100
SCD1  .......... .......... .......... .......... ..........
SCD3  CACAGTGCCC CACATGCCGG CTGTGGGAAT CCAGTTTCTG GCCAGTTCCC
SCD2  CTGCGATTTT AGGCGTCCTC TCATTTTCTA TCCTTATCTC CGCCCGCGGC
SCD4  TGGTGGCATC GAGGGCCCAC TGAGCATGGG TCCTGGGCTT AGCTCTTCTC

      101                                                150
SCD1  .......... .......... .......... .......... ..........
SCD3  GGTAGCGCGG GCCTAGAAAC CTGCTCAGCA TCGCTGCAGG GTCTCAATAC
SCD2  TGCCCTGGCC AGCCAGTTTT TTGATTTTTA TCTTGGTCAT TGATCAATAT
SCD4  AACTGCTGTC CTCAGCTTAA AAGGGGATAA ATGAAACCAA CTCTC.TGCT

      151                                                200
SCD1  .......... ......ACAG CCAGACCGGG CTGAACACCC ATCCCGAGAG
SCD3  TCAGGAATTC AGCGGCCGCC CGGGCAGGTG CTGAACACCC ATCCCGAGAG
SCD2  AACGAACCTA AGGATATCAG GACATTAATA CCCCACTGCC AGCTCTGGCC
SCD4  GCTTTAGCAG AGGACATGGA GAAACCCGGA CCCCACGATC ACATCTGGAC

      201                                                250
SCD1  TCAGGAGGGC AG..GTTTCC AAGCGCAGTT CCGCCACTCG CCTACACCAA
SCD3  TCAGGAGGGC AG..GTTTCC AAGCGCAGTT CCGCCACTCG CCTACACCAA
SCD2  CAGAGCTTGT ACGCGCAGCG GGCTGCAGAA ACTTAGTCAT AGCACCCTTT
SCD4  CAGAGAGTAT TGCAAATCCA GAAAACAGGA TCTGCAACAG AAGCCTCCTC

      251                                                300
SCD1  CGGGCTCCGG AACCGAAGTC CACGCTCGAT CTCAGCACTG GGAAAGTGAG
SCD3  CGGGCTCCGG AACCGAAGTC CACGCTCGAT CTCAGCACTG GGAAAGTGAG
SCD2  GTCGCTGAGG TCTGAAGCTC GCTGCACGTT CTCATCCCTG GGAACGTGAC
SCD4  TGCCCTGCAG CCCCAA..AC GCCACAACTT TAAATCCTTG GAAGATCTTC

      301                                                350
SCD1  GCGAGCAACT GACTATCATC ATGCCGGCCC ACATGCTCC. ..AAGAGATC
SCD3  GCGAGCAACT GACTATCATC ATGCCGGCCC ACATGCTCC. ..AAGAGATC
SCD2  CCCAGCATCC GAC.GCCAAG ATGCCGGCCC ACATACTGC. ..AAGAGATC
SCD4  CCGGCCTCCA AGA.ACCAGC ATGCCAGGGC ACCTGCTGCA AGAAGAGATG

      351                                                400
SCD1  TCCAGTTCTT ACACGACCAC CACCACCATC ACTGCACCTC CCTCCGGA..
SCD3  TCCAGTTCTT ACACGACCAC CACCACCATC ACTGCACCTC CCTCCGGA..
SCD2  TCTGGCGCTT ACTCAGCCAC CACCACAATC ACAGCGCCAC CTTCTGGGGG
SCD4  ACGCGTTCGT ACACGACCAC CACCACCATC ACAGCGCCTC CCTCTGGAAG
```

Fig. 4 (cont.)

```
          401                                                 450
SCD1  .......AAT GAACGAGAGA AGGTGAAGAC AGTGCCCCTC CACCTGGAAG
SCD3  .......AAT GAACGAGAGA AGGTGAAGAC GGTGCCCCTC CACCTGGAAG
SCD2  ACAGCAGAAT GGAGGCGAGA AGTTTGAAAA GAGTTCTCAC CACTGGGGAG
SCD4  CCTGCAGAAT GGACGAGAGA AGGTGAAGAC GGTGCCCCTC TACCTGGAAG

          451                                                 500
SCD1  AAGACATCCG TCCTGAAATG AAAGAAGATA TTCACGACCC CACCTATCAG
SCD3  AAGACATCCG TCCTGAAATG AAAGAAGATA TTCACGACCC CACCTATCAG
SCD2  CAGATGTTCG CCCTGAACTA AAAGATGATC TATATGACCC CACCTATCAG
SCD4  AAGACATCCG TCCTGAAATG AAAGAAGATA TATACGACCC CACCTATCAG

          501                                                 550
SCD1  GATGAGGAGG GACCCCCGCC CAAGCTGGAG TACGTCTGGA GGAACATCAT
SCD3  GATGAGGAGG GACCCCCGCC CAAGCTGGAG TACGTCTGGA GGAACATCAT
SCD2  GATGATGAGG GGCCCCCGCC CAAGCTGGAG TACGTCTGGA GGAACATCAT
SCD4  GATGAGGAGG GGCCCCCGCC CAAGCTGGAG TACGTCTGGA GGAACATCAT

          551                                                 600
SCD1  TCTCATGGTC CTGCTGCACT TGGGAGGCCT GTACGGGATC ATACTGGTTC
SCD3  TCTCATGGTC CTGCTGCACT TGGGAGGCCT GTACGGGATC ATACTGGTTC
SCD2  TCTCATGGCC CTGCTGCATT TGGGAGCCTT GTACGGGATC ACACTGGTTC
SCD4  TCTCATGGCC CTGCTGCACG TGGGAGCCCT GTACGGGATC ACACTGGTTC

          601                                                 650
SCD1  CCTCCTGCAA GCTCTACACT GCCCTCTTCG GGATTTTCTA CTACATGACC
SCD3  CCTCCTGCAA GCTCTACACC TGCCTCTTCG GGATTTTCTA CTACATGACA
SCD2  CCTCCTGCAA GCTCTACACC TGTCTCTTCG CGTATTTGTA CTATGTAATC
SCD4  CCTCCTGCAA GCTCTACACC TGCCTCTTCG CGTTTGTCTA CTATGTGATC

          651                                                 700
SCD1  AGCGCTCTGG GCATCACAGC CGGGGCTCAT CGCCTCTGGA GCCACAGAAC
SCD3  AGCGCTCTGG GCATCACAGC CGGGGCTCAT CGCCTCTGGA GCCACAGAAC
SCD2  AGCGCCCTGG GCATCACAGC CGGGGCTCAT CGCCTGTGGA GCCACAGAAC
SCD4  AGTATTGAGG GCATTGGAGC CGGAGTCCAT CGCCTGTGGA GCCACAGAAC

          701                                                 750
SCD1  TTACAAGGCT CGGCTGCCCC TGCGGATCTT CCTTATCATT GCCAACACCA
SCD3  TTACAAGGCA CGGCTGCCCC TGCGAATCTT CCTTATCATT GCCAACACCA
SCD2  ATACAAGGCA CGGCTGCCCC TGAGGCTCTT CCTCATCATT GCCAACACCA
SCD4  GTACAAGGCA CGCCTGCCCC TGCGGATCTT CCTCATCATT GCCAACACCA

          751                                                 800
SCD1  TGGCGTTCCA GAATGACGTG TACGAATGGG CCCGAGATCA CCGCGCCCAC
SCD3  TGGCGTTCCA GAATGACGTG TACGAATGGG CCCGAGATCA CCGCGCCCAC
SCD2  TGGCGTTCCA GAATGACGTG TATGAATGGG CCCGAGATCA CCGCGCCCAC
SCD4  TGGCGTTCCA GAATGACGTG TATGAATGGG CCCGAGATCA CCGAGCCCAC
```

Fig. 4 (con't)

```
          801                                                       850
SCD1  CACAAGTTCT CAGAAACACA CGCCGACCCT CACAATTCCC GCCGTGGCTT
SCD3  CACAAGTTCT CAGAAACACA CGCCGACCCT CACAATTCCC GCCGTGGCTT
SCD2  CACAAGTTCT CAGAAACACA CGCCGACCCT CACAATTCCC GCCGTGGCTT
SCD4  CACAAGTTCT CAGAAACACA CGCCGACCCT CACAATTCCC GCCGTGGCTT

          851                                                       900
SCD1  CTTCTTCTCT CACGTGGGTT GGCTGCTTGT GCGCAAACAC CCGGCTGTCA
SCD3  CTTCTTCTCT CACGTGGGTT GGCTGCTTGT GCGCAAACAC CCGGCTGTCA
SCD2  CTTTTTCTCT CACGTGGGTT GGCTGCTTGT GCGCAAACAC CCGGCTGTCA
SCD4  CTTCTTCTCT CACGTGGGTT GGCTGCTTGT GCGCAAACAC CCGGCTGTCA

          901                                                       950
SCD1  AAGAGAAGGG CGGAAAACTG GACATGTCTG ACCTGAAAGC CGAGAAGCTG
SCD3  AAGAGAAGGG CGGAAAACTG GACATGTCTG ACCTGAAAGC CGAGAAGCTG
SCD2  AAGAGAAGGG CGGAAAACTG GACATGTCTG ACCTGAAAGC CGAGAAGCTG
SCD4  AAGAGAAGGG CGGAAAACTG GACATGTCTG ACCTGAAAGC CGAGAAGCTG

          951                                                      1000
SCD1  GTGATGTTCC AGAGGAGGTA CTACAAGCCC GGCCTCCTGC TGATGTGCTT
SCD3  GTGATGTTCC AGAGGAGGTA CTACAAGCCC GACCTCCTGC TGATGTGCTT
SCD2  GTGATGTTCC AGAGGAGGTA CTACAAGCCC GACCTCCTGC TGATGTGCTT
SCD4  GTGATGTTCC AGAGGAGGTA CTACAAGCCT GGCATTCTGC TGATGTGCTT

         1001                                                      1050
SCD1  CATCCTGCCC ACGCTGGTGC CCTGGTACTG CTGGGGCGAG ACTTTTGTAA
SCD3  CATCCTGCCC ACGCTGGTGC CCTGGTACTG CTGGGGCGAG ACTTTTGTAA
SCD2  CGTCCTGCCC ACGCTGGTGC CCTGGTACTG CTGGGGCGAG ACTTTTGTAA
SCD4  CATCCTGCCC ACGCTGGTGC CCTGGTACTG CTGGGGCGAG ACTTTTCTAA

         1051                                                      1100
SCD1  ACAGCCTGTT CGTTAGCACC TTCTTGCGAT ACACTCTGGT GCTCAACGCC
SCD3  ACAGCCTGTT CGTTAGCACC TTCTTGCGAT ACACTCTGGT GCTCAACGCC
SCD2  ACAGCCTGTG CGTTAGCACC TTCTTGCGAT ACGCCGTGGT GCTCAACGCC
SCD4  ACAGTTTTTA TGTTGCCACT TTACTGAGAT ACGCTGTGGT GCTCAACGCC

         1101                                                      1150
SCD1  ACCTGGCTGG TGAACAGTGC CGCGCATCTC TATGGATATC GCCCCTACGA
SCD3  ACCTGGCTGG TGAACAGTGC CGCGCATCTC TATGGATATC GCCCCTACGA
SCD2  ACCTGGCTGG TGAACAGTGC CGCCCACCTC TACGGATATC GCCCCTACGA
SCD4  ACTTGGCTGG TGAACAGTGC CGCCCACCTC TACGGGTATC GCCCCTACGA

         1151                                                      1200
SCD1  CAAGAACATT CAATCCCGGG AGAATATCCT GGTTTCCCTG GGTGCCGTGG
SCD3  CAAGAACATT CAATCCCGGG AGAATATCCT GGTTTCCCTG GGTGCCGTGG
SCD2  CAAGAACATT AGGTCTCGGG AGAACATCTT GGTTTCCATG GGAGCTGTGG
SCD4  TAAGAACATC GATCCCCGGC AGAATGCCCT GGTTTCCTTG GGAAGTATGG
```

Fig. 4 (cont.)

```
          1201                                                       1250
SCD1  GCGAGGGCTT CCACAACTAC CACCACACCT TCCCCTTCGA CTACTCTGCC
SCD3  .......... .......... .......... .......... ..........
SCD2  GCGAGCGCTT CCACAACTAC CACCACGCCT TCCCCTACGA CTACTCTGCC
SCD4  GCGAGGGCTT CCACAACTAC CACCATGCCT TCCCCTACGA CTACTCTGCC

          1251                                                       1300
SCD1  AGTGAGTACC GCTGGCACAT CAACTTCACC ACGTTCTTCA TCGACTGCAT
SCD3  .......... .......... .......... .......... ..........
SCD2  AGTGAGTACC GCTGGCACAT CAACTTCACC ACGTTCTTCA TCGATTGCAT
SCD4  AGTGAGTACC GCTGGCACAT CAACTTCACC ACGTTCTTCA TCGACTGCAT

          1301                                                       1350
SCD1  GGCTGCCCTG GGCCTGGCTT ACGACCGGAA GAAAGTTTCT AAGGCTACTG
SCD3  .......... .......... .......... .......... ..........
SCD2  GGCTCTCCTG GGCCTGGCTT ACGACCGGAA GAGAGTGTCC AGGGCTGCTG
SCD4  GGCTGCACTG GGCCTGGCTT ACGACCGGAA GAGAGTGTCC AAGGCCACTG

          1351                                                       1400
SCD1  ICTTAGCCAG GATTAAGAGA ACTGGAGACG GGAGTCACAA GAGTAGCTGA
SCD3  .......... .......... .......... .......... ..........
SCD2  TCTTGGCCAG GATTAAGAGA ACTGGAGACG GAAGCTGCAA GAGCGGCTGA
SCD4  TCTTAGCCAG GATTAAGAGA ACTGGAGACG GGAGTCACAA GAGTGGCTGA

          1401                                                       1450
SCD1  GCTTTGGGCT TCTGAGTTCC TGTTTCAAAC GTTTTCTGGC AGAGATTTAA
SCD3  .......... .......... .......... .......... ..........
SCD2  GTGTGGGGTC TTGCAGTTCC TGT..... .......... ..........
SCD4  ATTTGGAGTC AGTCTATTCC AAAAGCCAGC TGGATAGGGG TTTAATAATG

          1451                                                       1500
SCD1  TATTCTGTTG ATTAACTAAC AACTGGATAT TGCTATCGGG GTGTTAATGA
SCD3  .......... .......... .......... .......... ..........
SCD2  .......... .......... .......... .......... ..........
SCD4  TTTTTTCAAA TACCGAAAAG AAGCACCCAT GTTGTATAGT GTCCTACTTC

          1501                                                       1550
SCD1  TGCATTTAAC CTATTCCGGT ACAGTATTCT TATAAAATGA GAAAGCTTTG
SCD3  .......... .......... .......... .......... ..........
SCD2  .......... .......... .......... .......... ..........
SCD4  AAGACAATAT TCTTGTAAAA TATTCAAATA TTAAAAGACC AAAACTTTCT

          1551                                                       1600
SCD1  ATCACGTTTT GAGGTAATAA ATATTTTATT TAGCTAGGAT TAACCATGCC
SCD3  .......... .......... .......... .......... ..........
SCD2  .......... .......... .......... .......... ..........
SCD4  TTTATGATGC TAAAAAAAAA AAAAAAAAA. .......... ..........
```

Fig. 5 **Deduced Protein Sequence (289 amino acids) from Mouse Skin SCD3 cDNA (upto the end of exon 5).**

```
  1  MPAHMLQEIS SSYTTTTTII APPSGNEREK VKTVPLHLEE DIRPEMKEDI

 51  HDPTYQDEEG PPPKLEYVWR NIILMVLLHL GGLYGIILVP SCKLYTCLFG

101  IFYYMTSALG ITAGAHRLWS HRTYKARLPL RIFLIIANTM AFQNDVYEWA

151  RDHRAHHKFS ETHADPHNSR RGFFFSHVGW LLVRKHPAVK EKGGKLDMSD

201  LKAEKLVMFQ RRYYKPGLLL MCFILPTLVP WYCWGETFVN SLFVSTFLRY

251  TLVLNATWLV NSAAHLYGYR PYDKNIQSRE NILVSLGAV
```

Fig. 6 **Complete Protein Coding Sequence of Mouse Skin SCD4 (359 amino acids) Deduced from its cDNA Sequence.**

```
  1  MPGHLLQEEM TPSYTTTTTI TAPPSGSLQN GREKVKTVPL YLEEDIRPEM
 51  KEDIYDPTYQ DEEGPPPKLE YVWRNIILMA LLHVGALYGI TLVPSCKLYT
101  CLFAFVYYVI SIEGIGAGVH RLWSHRTYKA RLPLRIFLII ANTMAFQNDV
151  YEWARDHRAH HKFSETHADP HNSRRGFFFS HVGWLLVRKH PAVKEKGGKL
201  DMSDLKAEKL VMFQRRYYKP GILLMCFILP TLVPWYCWGE TFLNSFYVAT
251  LLRYAVVLNA TWLVNSAAHL YGYRPYDKNI DPRQNALVSL GSMGEGFHNY
301  HHAFPYDYSA SEYRWHINFT TFFIDCMAAL GLAYDRKRVS KATVLARIKR
351  TGDQGSHKSG*
```

Fig. 7 **Comparison of Protein Sequences From Four Mouse SCD Genes**

```
          1                                                  50
SCD1  MPAHMLQ.EI SSSYTTTTTI TAPPSG...N EREKVKTVPL HLEEDIRPEM
SCD3  MPAHMLQ.EI SSSYTTTTTI TAPPSG...N EREKVKTVPL HLEEDIRPEM
SCD4  MPGHLLQEEM TPSYTTTTTI TAPPSGSLQN GREKVKTVPL YLEEDIRPEM
SCD2  MPAHILQ.EI SGAYSATTTI TAPPSGGQQN GGEKFEKSSH HWGADVRPEL

         51                                                 100
SCD1  KEDIHDPTYQ DEEGPPPKLE YVWRNIILMV LLHLGGLYGI ILVPSCKLYT
SCD3  KEDIHDPTYQ DEEGPPPKLE YVWRNIILMV LLHLGGLYGI ILVPSCKLYT
SCD4  KEDIYDPTYQ DEEGPPPKLE YVWRNIILMA LLHVGALYGI ILVPSCKLYT
SCD2  KDDLYDPTYQ DDEGPPPKLE YVWRNIILMA LLHLGALYGI ILVPSCKLYT

         101                                                150
SCD1  ALFGIFYYMT SALGITAGAH RLWSHRTYKA RLPLRIFLII ANTMAFQNDV
SCD3  CLFGIFYYMT SALGITAGAH RLWSHRTYKA RLPLRIFLII ANTMAFQNDV
SCD4  CLFAFVYYVI SIEGIGAGVH RLWSHRTYKA RLPLRIFLII ANTMAFQNDV
SCD2  CLFAYLYYVI SALGITAGAH RLWSHRTYKA RLPLRLFLII ANTMAFQNDV

         151                                                200
SCD1  YEWARDHRAH HKFSETHADP HNSRRGFFFS HVGWLLVRKH PAVKEKGGKL
SCD3  YEWARDHRAH HKFSETHADP HNSRRGFFFS HVGWLLVRKH PAVKEKGGKL
SCD4  YEWARDHRAH HKFSETHADP HNSRRGFFFS HVGWLLVRKH PAVKEKGGKL
SCD2  YEWARDHRAH HKFSETHADP HNSRRGFFFS HVGWLLVRKH PAVKEKGGKL

         201                                                250
SCD1  DMSDLKAEKL VMFQRRYYKP GLLLMCFILP TLVPWYCWGE TFVNSLFVST
SCD3  DMSDLKAEKL VMFQRRYYKP GLLLMCFILP TLVPWYCWGE TFVNSLFVST
SCD4  DMSDLKAEKL VMFQRRYYKP GILLMCFILP TLVPWYCWGE TFLNSFYVAT
SCD2  DMSDLKAEKL VMFQRRYYKP DLLLMCFVLP TLVPWYCWGE TFVNSLCVST

         251                                                300
SCD1  FLRYTLVLNA TWLVNSAAHL YGYRPYDKNI QSRENILVSL GAVGEGFHNY
SCD3  FLRYTLVLNA TWLVNSAAHL YGYRPYDKNI QSRENILVSL GAV.......
SCD4  LLRYAVVLNA TWLVNSAAHL YGYRPYDKNI DPRQNALVSL GSMGEGFHNY
SCD2  FLRYAVVLNA TWLVNSAAHL YGYRPYDKNI SSRENILVSM GAVGERFHNY
```

Fig. 7 (cont.)

```
      301                                                        350
SCD1  HHTFPFDYSA SEYRWHINFT TFFIDCMAAL GLAYDRKKVS KATVLARIKR
SCD3  .......... .......... .......... .......... ..........
SCD4  HHAFPYDYSA SEYRWHINFT TFFIDCMAAL GLAYDRKRVS KATVLARIKR
SCD2  HHAFPYDYSA SEYRWHINFT TFFIDCMALL GLAYDRKRVS RAAVLARIKR

      351
SCD1  TGDGSHKSS*
SCD3  ..........
SCD4  TGDGSHKSG.
SCD2  TGDGSCKSG*
```

Fig. 8 Human Skin SCD cDNA

GGGGCTGAGGAAATACCGGACACGGTCACCCGTTGCCAGCTCTAGCCTTTAAATTCCCGG
CTCGGGGACCTCCACGCACCGCGGCTAGCGCCGACAACCAGCTAGCGTGCAAGGCGCCGC
GGCTCAGCGCGTACCGGCGGGCTTCGAAACCGCAGTCCTCCGGCGACCCCGAACTCCGCT
CCGGAGCCTCAGCCCCCTGGAAAGTGATCCCGGCATCCGAGAGCCAAGATGCCGGCCCAC
TTGCTGCAGGACGATATCTCTAGCTCCTATACCACCACCACCACCATTACAGCGCCTCCC
TCCAGGGTCCTGCAGAATGGAGGAGATAAGTTGGAGACGATGCCCCTCTACTTGGAAGAC
GACATTCGCCCTGATATAAAAGATGATATATATGACCCCACCTACAAGGATAAGGAAGGC
CCAAGCCCCAAGGTTGAATATGTCTGGAGAAACATCATCCTTATGTCTCTGCTACACTTG
GGAGCCCTGTATGGGATCACTTTGATTCCTACCTGCAAGTTCTACACCTGGCTTTGGGGG
GTATTCTACTATTTTGTCAGTGCCCTGGGCATAACAGCAGGAGCTCATCGTCTGTGGAGC
CACCGCTCTTACAAAGCTCGGCTGCCCCTACGGCTCTTTCTGATCATTGCCAACACAATG
GCATTCCAGAATGATGTCTATGAATGGGCTCGTGACCACCGTGCCCACCACAAGTTTTCA
GAAACACATGCTGATCCTCATAATTCCCGACGTGGCTTTTTCTTCTCTCACGTGGGTTGG
CTGCTTGTGCGCAAACACCCAGCTGTCAAAGAGAAGGGGAGTACGCTAGACTTGTCTGAC
CTAGAAGCTGAGAAACTGGTGATGTTCCAGAGGAGGTACTACAAACCTGGCTTGCTGCTG
ATGTGCTTCATCCTGCCCACGCTTGTGCCCTGGTATTTCTGGGGTGAAACTTTTCAAAAC
AGTGTGTTCGTTGCCACTTTCTTGCGATATGCTGTGGTGCTTAATGCCACCTGGCTGGTG
AACAGTGCTGCCCACCTCTTCGGATATCGTCCTTATGACAAGAACATTAGCCCCCGGGAG
AATATCCTGGTTTCACTTGGAGCTGTGGGTGAGGGCTTCCACAACTACCACCACTCCTTT
CCCTATGACTACTCTGCCAGTGAGTACCGCTGGCACATCAACTTCACCACATTCTTCATT
GATTGCATGGCCGCCCTCGGTCTGGCCTATGACCGGAAGAAAGTCTCCAAGGCCGCCATC
TTGGCCAGGATTAAAAGAACCGGAGATGGAAACTACAAGAGTGGCTGAGTTTGGGGTCCC
TCAGGTTTCGTTTTCAAAAACCAGCCAGGCAGAGGTTTTAATGTCTGTTTATTAACTACT
GAATAATGCTACCAGGATGCTAAAGATGATGATGTTAACCCATTCCAGTACAGTATTCTT
TTAAAATTCAAAAGTATTGAAAGCCAACAACTCTGCCTTTATGATGCTAAGCTGATATTA
TTTCTTCTCTTATCCTCTCTCTCTTCTAGGCCCATTGTCCTCCTTTTCACTTTATTGCTA
TCGCCCTCCTTTCCCTTATTGCCTCCCAGGCAAGCAGCTGGTCAGTCTTTGCTCAGTGTC
CAGCTTCCAAAGCCTAGACAACCTTTCTGTAGCCTAAAACGAATGGTCTTTGCTCCAGAT
AACTCTCTTTCCTTGAGCTGTTGTGAGCTTTGAAGTAGGTGGCTTGAGCTAGAGATAAAA
CAGAATCTTCTGGGTAGTCCCCTGTTGATTATCTTCAGCCCAGGCTTTTGCTAGATGGAA
TGGAAAAGCAACTTCATTTGACACAAAGCTTCTAAAGCAGGTAAATTGTCGGGGGAGAGA
GTTAGCATGTATGAATGTAAGGATGAGGGAAGCGAAGCAAGAGGAACCTCTCGCCATGAT
CAGACATACAGCTGCCTACCTAATGAGGACTTCAAGCCCCACCACATAGCATGCTTCCTT
TCTCTCCTGGCTCGGGG

Fig. 8 (cont.)

Human Skin Protein Sequence

MPAHLLQDDISSSYTTTTTITAPPSRVLQNGGDKLETMPLYLEDDIRPDIKDDIYDP
TYKDKEGPSPKVEYVWRNIILMSLLHLGALYGITLIPTCKFYTWLWGVFYYFVSAL
GITAGAHRLWSHRSYKARLPLRLFLIIANTMAFQNDVYEWARDHRAHHKFSETHA
DPHNSRRGFFFSHVGWLLVRKHPAVKEKGSTLDLSDLEAEKLVMFQRRYYKPG
LLLMCFILPTLVPWYFWGETFQNSVFVATFLRYAVVLNATWLVNSAAHLFGYRPY
DKNISPRENILVSLGAVGEGFHNYHHSFPYDYSASEYRWHINFTTFFIDCMAALG
LAYDRKKVSKAAILARIKRTGDGNYKSG*

Fig. 9 Comparison between human skin SCD cDNA & human liver SCD cDNA

```
skin   1 GGGGCTGAGGAAATACCGGACACGGTCACCCGTTGCCAGCTCTAGCCTTT 50
liver  1 ..................GACGGTCACCCGTTGCCAGCTCTAGCCTTT 30

      51 AAATTCCCGGCTCGGGGACCTCCACGCACCGCGGCTAGCGCCGACAACCA 100
      31 AAATTCCCGGCTCGGGGACCTCCACGCACCGCGGCTAGCGCCGACAACCA 80

     101 GCTAGCGTGCAAGGCGCCGCGGCTCAGCGCGTACCGGCGGGCTTCGAAAC 150
      81 GCTAGCGTGCAAGGCGCCGCGGCTCAGCGCGTACCGGCGGGTTTCGAAAC 130

     151 CGCAGTCCTCCGGCGACCCCGAACTCCGCTCCGGAGCCTCAGCCCCCTGG 200
     131 CGCAGTCCTCCGGCGACCCCGAACTCCGCTCCGGAGCCTCAGCCCCCTGG 180

     201 AAAGTGATCCCGGCATCCGAGAGCCAAGATGCCGGCCCACTTGCTGCAGG 250
     181 AAAGTGATCCCGGCATCCGAGAGCCAAGATGCCGGCCCACTTGCTGCAGG 230

     251 ACGATATCTCTAGCTCCTATACCACCACCACCACCATTACAGCGCCTCCC 300
     231 ACGATATCTCTAGCTCCTATACCACCACCACCACCATTACAGCGCCTCCT 280

     301 TCCAGGGTCCTGCAGAATGGAGGAGATAAGTTGGAGACGATGCCCCTCTA 350
     281 CCAGGGGTCCTGCAGAATGGAGGAGATAAGTTGGAGACGATGCCCCTCTA 330

     351 CTTGGAAGACGACATTCGCCCTGATATAAAAGATGATATATATGACCCCA 400
     331 CTTGGAAGACGACATTCGCCCTGATATAAAAGATGATATATATGACCCCA 380

     401 CCTACAAGGATAAGGAAGGCCCAAGCCCCAAGGTTGAATATGTCTGGAGA 450
     381 CCTACAAGGATAAGGAAGGCCCAAGCCCCAAGGTTGAATATGTCTGGAGA 430

     451 AACATCATCCTTATGTCTCTGCTACACTTGGGAGCCCTGTATGGGATCAC 500
     431 AACATCATCCTTATGTCTCTGCTACACTTGGGAGCCCTGTATGGGATCAC 480

     501 TTTGATTCCTACCTGCAAGTTCTACACCTGGCTTTGGGGGGTATTCTACT 550
     481 TTTGATTCCTACCTGCAAGTTCTACACCTGGCTTTGGGGGGTATTCTACT 530

     551 ATTTTGTCAGTGCCCTGGGCATAACAGCAGGAGCTCATCGTCTGTGGAGC 600
     531 ATTTTGTCAGTGCCCTGGGCATAACAGCAGGAGCTCATCGTCTGTGGAGC 580
```

```
601  CACCGCTCTTACAAAGCTCGGCTGCCCCTACGGCTCTTTCTGATCATTGC  650
     ||||||||||||||||||||||||||||||||||||||||||||||||||
581  CACCGCTCTTACAAAGCTCGGCTGCCCCTACGGCTCTTTCTGATCATTGC  630

651  CAACACAATGGCATTCCAGAATGATGTCTATGAATGGGCTCGTGACCACC  700
     ||||||||||||||||||||||||||||||||||||||||||||||||||
631  CAACACAATGGCATTCCAGAATGATGTCTATGAATGGGCTCGTGACCACC  680

701  GTGCCCACCACAAGTTTTCAGAAACACATGCTGATCCTCATAATTCCCGA  750
     ||||||||||||||||||||||||||||||||||||||||||||||||||
681  GTGCCCACCACAAGTTTTCAGAAACACATGCTGATCCTCATAATTCCCGA  730

751  CGTGGCTTTTTCTTCTCTCACGTGGGTTGGCTGCTTGTGCGCAAACACCC  800
     ||||||||||||||||||||||||||||||||||||||||||||||||||
731  CGTGGCTTTTTCTTCTCTCACGTGGGTTGGCTGCTTGTGCGCAAACACCC  780

801  AGCTGTCAAAGAGAAGGGGAGTACGCTAGACTTGTCTGACCTAGAAGCTG  850
     ||||||||||||||||||||||||||||||||||||||||||||||||||
781  AGCTGTCAAAGAGAAGGGGAGTACGCTAGACTTGTCTGACCTAGAAGCTG  830

851  AGAAACTGGTGATGTTCCAGAGGAGGTACTACAAACCTGGCTTGCTGCTG  900
     |||||||||||||||||||||||||||||||||||||||||||||| ||
831  AGAAACTGGTGATGTTCCAGAGGAGGTACTACAAACCTGGCTTGCTGATG  880

901  ATGTGCTTCATCCTGCCCACGCTTGTGCCCTGGTATTTCTGGGGTGAAAC  950
     ||||||||||||||||||||||||||||||||||||||||||||||||||
881  ATGTGCTTCATCCTGCCCACGCTTGTGCCCTGGTATTTCTGGGGTGAAAC  930

951  TTTTCAAAACAGTGTGTTCGTTGCCACTTTCTTGCGATATGCTGTGGTGC  1000
     ||||||||||||||||||||||||||||||||||||||||||||||||||
931  TTTTCAAAACAGTGTGTTCGTTGCCACTTTCTTGCGATATGCTGTGGTGC  980

1001 TTAATGCCACCTGGCTGGTGAACAGTGCTGCCCACCTCTTCGGATATCGT  1050
     ||||||||||||||||||||||||||||||||||||||||||||||||||
981  TTAATGCCACCTGGCTGGTGAACAGTGCTGCCCACCTCTTCGGATATCGT  1030

1051 CCTTATGACAAGAACATTAGCCCCCGGGAGAATATCCTGGTTTCACTTGG  1100
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1031 CCTTATGACAAGAACATTAGCCCCCGGGAGAATATCCTGGTTTCACTTGG  1080

1101 AGCTGTGGGTGAGGGCTTCCACAACTACCACCACTCCTTTCCCTATGACT  1150
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1081 AGCTGTGGGTGAGGGCTTCCACAACTACCACCACTCCTTTCCCTATGACT  1130

1151 ACTCTGCCAGTGAGTACCGCTGGCACATCAACTTCA CACATTCTTCATT  1200
     ||||||||||||||||||||||||||||||||||| ||||||||||||||
1131 ACTCTGCCAGTGAGTACCGCTGGCACATCAACTTCAACACATTCTTCATT  1180
```

Fig. 9 (cont.)

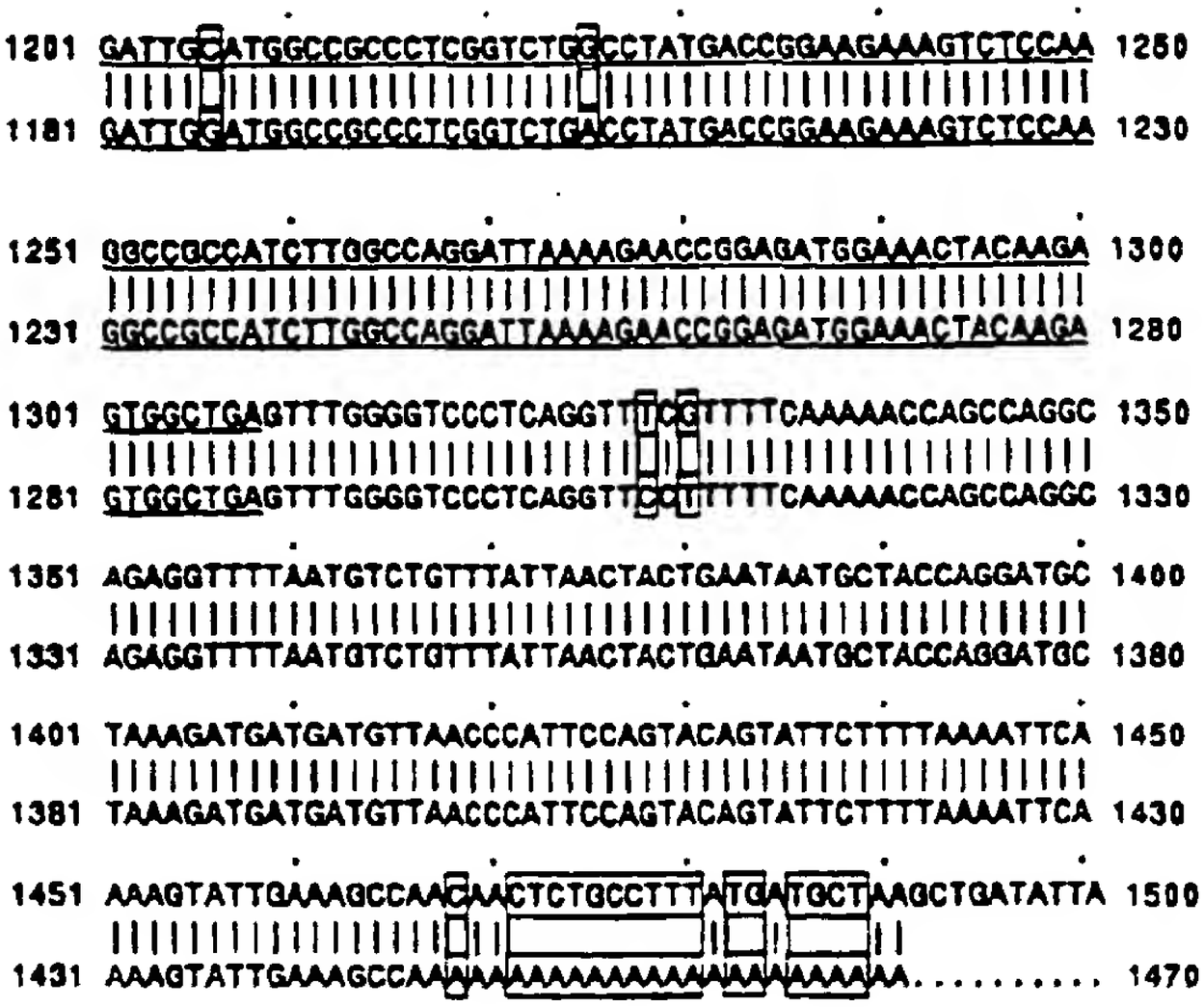
1201 GATTGCATGGCCGCCCTCGGTCTGGCCTATGACCGGAAGAAAGTCTCCAA 1250
1181 GATTGGATGGCCGCCCTCGGTCTGACCTATGACCGGAAGAAAGTCTCCAA 1230
1251 GGCCGCCATCTTGGCCAGGATTAAAAGAACCGGAGATGGAAACTACAAGA 1300
1231 GGCCGCCATCTTGGCCAGGATTAAAAGAACCGGAGATGGAAACTACAAGA 1280
1301 GTGGCTGAGTTTGGGGTCCCTCAGGTTTCGTTTTCAAAAACCAGCCAGGC 1350
1281 GTGGCTGAGTTTGGGGTCCCTCAGGTTCGTTTTTCAAAAACCAGCCAGGC 1330
1351 AGAGGTTTTAATGTCTGTTTATTAACTACTGAATAATGCTACCAGGATGC 1400
1331 AGAGGTTTTAATGTCTGTTTATTAACTACTGAATAATGCTACCAGGATGC 1380
1401 TAAAGATGATGATGTTAACCCATTCCAGTACAGTATTCTTTTAAAATTCA 1450
1381 TAAAGATGATGATGTTAACCCATTCCAGTACAGTATTCTTTTAAAATTCA 1430
1451 AAAGTATTGAAAGCCAACAACTCTGCCTTTATGATGCTAAGCTGATATTA 1500
1431 AAAGTATTGAAAGCCAA

Fig. 10 Comparison between human skin SCD cDNA and human adipose SCD cDNA

```
skin 201 AAAGTGATCCCGGCATCCGAGAGCCAAGATGCCGGCCCACTTGCTGCAGG 250
adip   1 ..............................GGCCCACATGCTCCAAG 17

     251 ACGATATCTCTAGCTCCTATACCACCACCACCACCATTACAGCGCCTCCC 300
      18 AGGATATCTCAAGCTCCTATACCACCACCACCACCATTACAGCGCCTCCT 67

     301 TCCAGGGTCCTGCAGAATGGAGGAGATAAGTTGGAGACGATGCCCCTCTA 350
      68 CCAGGGGTCCTGCAGAATGGAGGAGATAAGTTGGAGACGATGCCCCTCTA 117

     351 CTTGGAAGACGACATTCGCCCTGATATAAAAGATGATATATATGACCCCA 400
     118 CTTGGAAGACGACATTCGCCCTGATATAAAAGATGATATATACGACCCCA 167

     401 CCTACAAGGATAAGGAAGGCCCAAGCCCCAAGGTTGAATATGTCTGGAGA 450
     168 CCTACAAGGATAAGGAAGGCCCAAGCCCCAAGGTTGAATATGTCTGGAGA 217

     451 AACATCATCCTTATGTCTCTGCTACACTTGGGAGCCCTGTATGGGATCAC 500
     218 AACATCATCCTTATGTCTCTGCTACACTTGGGAGCCCTGTATGGGATCAC 267

     501 TTTGATTCCTACCTGCAAGTTCTACACCTGGCTTTGGGGGGTATTCTACT 550
     268 TTTGATTCCTACCTGCAAGTTCTACACCTGGCTTTGGGGGGTATTCTACT 317

     551 ATTTTGTCAGTGCCCTGGGCATAACAGCAGGAGCTCATCGTCTGTGGAGC 600
     318 ATTTTGTCAGTGCCCTGGGCATAACAGCAGGAGCTCATCGTCTGTGGAGC 367

     601 CACCGCTCTTACAAAGCTCGGCTGCCCCTACGGCTCTTTCTGATCATTGC 650
     368 CACCGCTCTTACAAAGCTCGGCTGCCCCTACGGCTCTTTCTGATCATTGC 417

     651 CAACACAATGGCATTCCAGAATGATGTCTATGAATGGGCTCGTGACCACC 700
     418 CAACACAATGGCATTCCAGAATGATGTCTATGAATGGGCTCGTGACCACC 467

     701 GTGCCCACCACAAGTTTTCAGAAACACATGCTGATCCTCATAATTCCCGA 750
     468 GTGCCCACCACAAGTTTTCAGAAACACATGCTGATCCTCATAATTCCCGA 517
```

Fig. 10 (cont.


751 CGTGGCTTTTTCTTCTCTCACGTGGGTTGGCTGCTTGTGCGCAAACACCC 800
518 CGTGGCTTTTTCTTCTCTCACGTGGGTTGGCTGCTTGTGCGCAAACACCC 567
801 AGCTGTCAAAGAGAAGGGGAGTACGCTAGACTTGTCTGACCTAGAAGCTG 850
568 AGCTGTCAAAGAGAAGGGGAGTACGCTAGACTTGTCTGACCTAGAAGCTG 617
851 AGAAACTGGTGATGTTCCAGAGGAGGTACTACAAACCTGGCTTGCTGCTG 900
618 AGAAACTGGTGATGTTCCAGAGGAGGTACTACAAACCCGGCTTGCTGATG 667
901 ATGTGCTTCATCCTGCCCACGCTTGTGCCCTGGTATTTTCTGGGGTGAAAC 950
668 ATGTGCTTCATCCTGCCCACGCTTGTGCCCTGGTACTGCTGGGGC..... 712

Fig. 11 Comparison of amino acid sequence of human liver, adipose, and skin SCD

```
          1                                                  50
liver     MPAHLLQDDI SSSYTTTTTI TAPPPGVLQN GGDKLETMPL YLEDDIRPDI
adipose   MPAHLLQDDI SSSYTTTTTI TAPPPGVLQN GGDKLETMPL YLEDDIRPDI
skin      MPAHLLQDDI SSSYTTTTTI TAPPSRVLQN GGDKLETMPL YLEDDIRPDI

          51                                                 100
          KDDIYDPTYK DKEGPSPKVE YVWRNIILMS LLHLGALYGI TLIPTCKFYT
          KDDIYDPTYK DKEGPSPKVE YVWRNIILMS LLHLGALYGI TLIPTCKFYT
          KDDIYDPTYK DKEGPSPKVE YVWRNIILMS LLHLGALYGI TLIPTCKFYT

          101                                                150
          WLWGVFYYFV SALGITAGAH RLWSHRSYKA RLPLRLFLII ANTMAFQNDV
          WLWGVFYYFV SALGITAGAH RLWSHRSYKA RLPLRLFLII ANTMAFQNDV
          WLWGVFYYFV SALGITAGAH RLWSHRSYKA RLPLRLFLII ANTMAFQNDV

          151                                                200
          YEWARDHRAH HKFSETHADP HNSRRGFFFS HVGWLLVRKH PAVKEKGSTL
          YEWARDHRAH HKFSETHADP HNSRRGFFFS HVGWLLVRKH PAVKEKGSTL
          YEWARDHRAH HKFSETHADP HNSRRGFFFS HVGWLLVRKH PAVKEKGSTL

          201                                                250
          DLSDLEAEKL VMFQRRYYKP GLLMMCFILP TLVPWYFWGE TFQNSVFVAT
          DLSDLEAEKL VMFQRRYYKP GLLMMCFILP TLVPWYCWG- ----------
          DLSDLEAEKL VMFQRRYYKP GLLLMCFILP TLVPWYFWGE TFQNSVFVAT

          251                                                300
          FLRYAVVLNA TWLVNSAAHL FGYRPYDKNI SPRENILVSL GAVGEGFHNY
          ---------- ---------- ---------- ---------- ----------
          FLRYAVVLNA TWLVNSAAHL FGYRPYDKNI SPRENILVSL GAVGEGFHNY

          301                                                350
          HHSFPYDYSA SEYRWHINFN TFFIDCMAAL GLAYDRKKVS KAAILARIKR
          ---------- ---------- ---------- ---------- ----------
          HHSFPYDYSA SEYRWHINFT TFFIDCMAAL GLAYDRKKVS KAAILARIKR

          351
          TGDGNYKSG
          ---------
          TGDGNYKSG
```

Fig. 12 cDNA Sequence Homology (5′ end) of the Two Mouse Skin SCD4 Variant Species

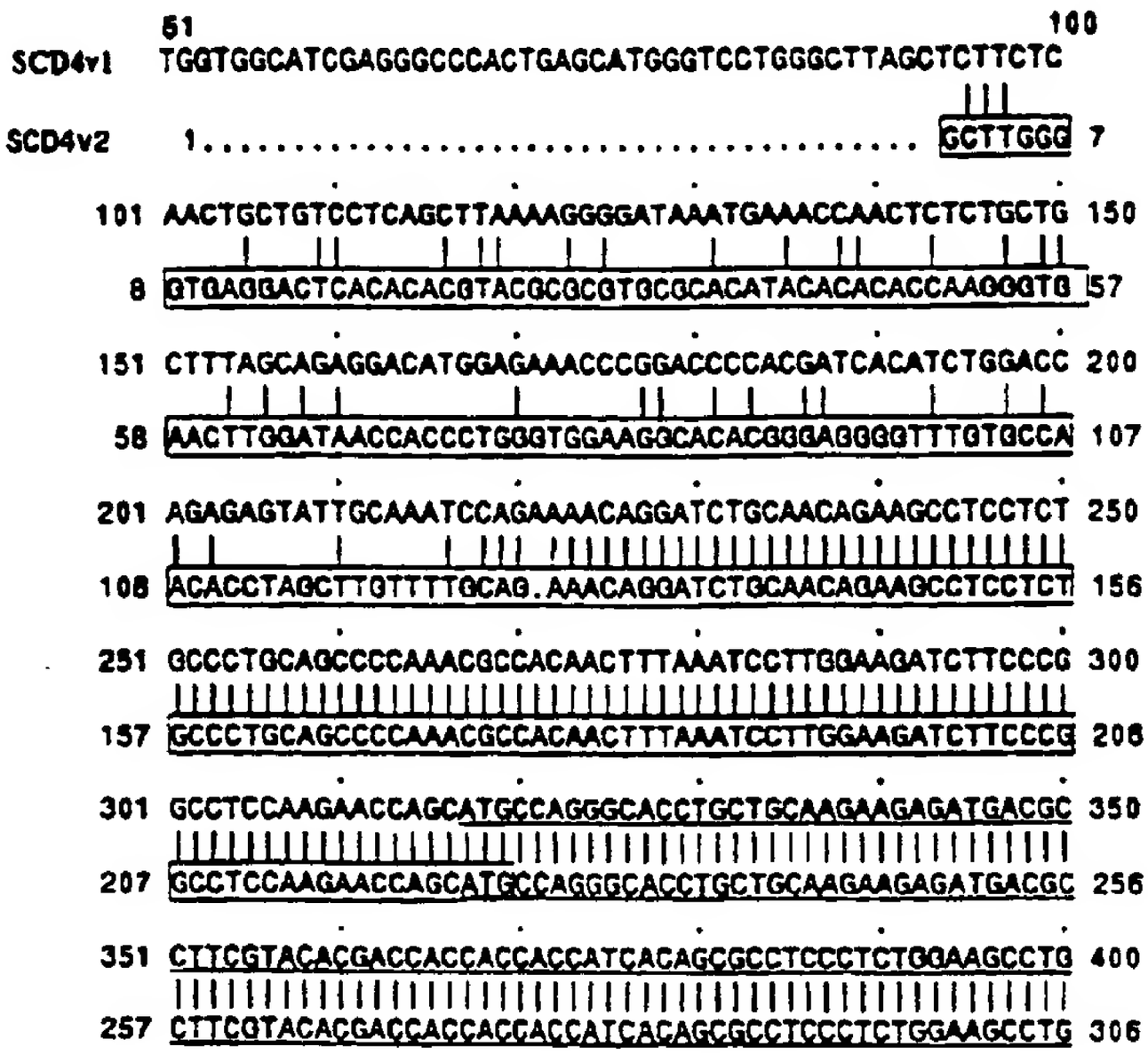

Fig. 13 **cDNA Sequence Homology (3' end) of the Two Mouse Skin SCD4 Variant Species**